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(71) Applicant (*for all designated States except US*): SRI INTERNATIONAL [US/US]; 333 Ravenswood Avenue, Menlo Park, CA 94025-3493 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): WEBSTER, Keith, A. [GB/US]; 3827 Grove Avenue, Palo Alto, CA 94303 (US). BISHOPRIC, Nanette, H. [US/US]; 3827 Grove Avenue, Palo Alto, CA 94303 (US). MURPHY, Brian [CA/US]; 395 Matadero Avenue, Palo Alto, CA 94306 (US). LADEROUTE, Keith, R. [CA/US]; Apartment 18, 750 Coleman Avenue, Menlo Park, CA 94025 (US). GREEN, Christopher, J. [US/US]; 2737 Topaz Drive, Novato, CA 94945 (US).

(74) Agent: SHOLTZ, Charles, K.; Dehlinger & Associates, P.O. Box 60850, Palo Alto, CA 94306-1546 (US).



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(54) Title: TISSUE SPECIFIC HYPOXIA REGULATED THERAPEUTIC CONSTRUCTS


(57) Abstract

Methods and compositions relating to chimeric genes containing (i) a tissue-specific promoter and (ii) a hypoxia response enhancer element, both of which are operably linked to a selected gene, such as a reporter gene, therapeutic gene (e.g., bcl-2, NOS, catalase and SOD), or deleterious gene are disclosed. Expression of the selected gene is enhanced in the target tissue under hypoxic conditions, such as conditions encountered during episodes of ischemia and reperfusion. The methods and compositions may be used as therapeutics and/or diagnostics.

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# TISSUE SPECIFIC HYPOXIA REGULATED THERAPEUTIC CONSTRUCTS

## FIELD OF THE INVENTION

This invention relates to chimeric genes (*e.g.*, carried on expression vectors) containing therapeutic genes whose expression is under the control of tissue specific and hypoxia response enhancer elements.

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## BACKGROUND OF THE INVENTION

Each year, over a half-million Americans die from heart attacks. Even more -- close to 700,000 -- have non-fatal heart attacks. For these surviving victims, a portion of the heart is usually damaged irreparably. Such cell death of cardiac tissue, called myocardial infarction,

is due in large part to tissue damage caused by ischemia and/or ischemia followed by reperfusion.

Similar ischemic damage may occur in many other tissues when the blood supply to the tissue is reduced or cut off. Stroke, deep vein thrombosis, pulmonary embolus, and renal failure are examples.

Surviving victims of ischemic episodes, such as heart attacks, are at substantially greater risk for subsequent episodes of ischemia, which in many cases prove debilitating or fatal. Thus, it would be desirable to have therapeutic methods and compositions by which survivors of heart attacks and other types of ischemic insults could lower the risk of tissue damage due to recurrent ischemic/reperfusion episodes.

## SUMMARY OF THE INVENTION

In one aspect, the invention includes a method for reducing ischemic injury to a cell exposed to hypoxic conditions. The method includes introducing into the cell a chimeric gene containing a hypoxia response element, a therapeutic gene, and a tissue-specific promoter operably linked to the therapeutic gene to control transcription of the therapeutic gene in the cell, where the element is effective to modulate expression of the therapeutic gene. Exposing the cell to hypoxic conditions enhances expression of the gene and expression of the gene is effective in reducing ischemic injury to the cell. The method may be applied to, for example, cardiac cells using a cardiac-specific promoter, kidney cells using a kidney-specific promoter, brain cells using a brain-specific promoter, and vascular endothelium cells using a vascular endothelium-specific promoter. The hypoxia response element may be selected from, for example, the erythropoietin HRE element (HREE1), muscle pyruvate kinase (PKM) HRE element, $\beta$-enolase (enolase 3; ENO3) HRE element, endothelin-1 (ET-1) HRE element and metallothionein II (MTII) HRE element. The therapeutic gene may be selected from, for example, nitric oxide synthase (NOS), B-cell leukemia/lymphoma 2 (bcl-2), superoxide dismutase (SOD) and catalase. In a preferred embodiment, the promoter is heterologous to said element.

In another aspect, the invention includes a chimeric gene, containing a hypoxia response element, a tissue-specific promoter heterologous to the element, and a therapeutic gene. The promoter is operably linked to the therapeutic gene and the element is effective to modulate expression of the therapeutic gene. The method may be used with a variety of cell types and corresponding promoters, for example, as identified above. Suitable cardiac-specific promoters include the $\alpha$-MHC$_{5.5}$ promoter, $\alpha$-MHC$_{86}$ promoter, and human cardiac actin

promoter. Suitable kidney-specific promoters include the renin promoter. Suitable brain-specific promoters include the aldolase C promoter and the tyrosine hydroxylase promoter. Suitable vascular endothelium-specific promoters include the Et-1 promoter and vonWillebrand factor promoter. Hypoxia response enhancer element useful with the method include HREE1, PKM HRE element, ENO3 HRE element and ET-1 HRE element. Exemplary therapeutic genes useful with the method include NOS, Bcl-2, SOD and catalase.

Another aspect of the present invention includes the above-described chimeric gene carried in an expression vector. The expression vector may be a plasmid, adenovirus vector, retrovirus vector, or the like.

In still another aspect, the invention includes a chimeric gene which contains a hypoxia response element, a tissue-specific promoter heterologous to the element, and a deleterious gene. The promoter is operably linked to the deleterious gene, and the element is effective to modulate expression of the deleterious gene. Suitable promoters include tumor-specific promoters, such as alpha fetoprotein (AFP) promoter. Suitable hypoxia response elements are as articulated above. Deleterious genes useful in this aspect include a viral thymidine kinase gene (tk), such as the herpes simplex virus (HSV) tk, and tumor necrosis factor (TNF).

In a related aspect, the invention includes a method of causing injury to a cell exposed to hypoxic conditions. The method includes introducing into the cell a vector containing a hypoxia response element, a deleterious gene, and a tissue-specific promoter operably linked to the gene and capable of controlling transcription of the gene in the cell. Exposing the cell to hypoxic conditions enhances expression of the gene, and expression of the gene is effective to cause injury to the cell. Promoters useful with this method include tumor-specific promoters such as the AFP promoter. Specific hypoxia response elements and deleterious genes useful with the method are also as identified above.

The invention also includes a chimeric gene which contains a hypoxia response element isolated from the metallothionein II promoter (e.g., an HRE contained in a fragment having the sequence represented as SEQ ID NO:35), a promoter and a heterologous gene. In one general embodiment, the heterologous gene is a therapeutic gene, as described above. In another general embodiment, the heterologous gene is a deleterious gene as described above (e.g., a DNA sequence encoding tumor necrosis factor).

The invention further includes a method of causing injury to a cell exposed to hypoxic conditions. The method includes introducing into the cell a vector containing a hypoxia response element isolated from the metallothionein II promoter (e.g., an HRE contained in a fragment having the sequence represented as SEQ ID NO:35), a promoter and a deleterious

gene (e.g., TNF). Exposing the cell to hypoxic conditions enhances expression of the deleterious gene, and expression of the gene is effective to cause injury to the cell.

The invention further includes a substantially isolated polynucleotide having a sequence corresponding to hypoxia response enhancer element(s) (HREE(s)) present in a control region of the muscle pyruvate kinase gene. The element may be derived from the promoter region, 5' untranslated region, or 3' untranslated region. In a related aspect, the invention includes an HRE element derived from a muscle pyruvate kinase gene.

Also included in the invention is a substantially isolated polynucleotide having a sequence corresponding to hypoxia response element(s) present in a control region of the endothelin-1 gene. The element may be derived from the promoter region, 5' untranslated region, or 3' untranslated region. In a related aspect, the invention includes an HRE element derived from an endothelin-1 gene.

Another aspect of the invention includes a substantially isolated polynucleotide having a sequence corresponding to hypoxia response element(s) present in a control region of the enolase 3 (ENO3) gene. The element may be derived from the promoter region, 5' untranslated region, or 3' untranslated region. In a related aspect, the invention includes an HRE element derived from an ENO3 gene. In another related aspect, the invention includes a hypoxia responsive element (HRE) contained in the region of the metallothionein II (MTAII) promoter corresponding to SEQ ID NO:35. In a preferred embodiment, the HRE element consists of a sequence derived from SEQ ID NO:35.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

## Brief Description of the Figures

Figures 1A and 1B show a schematic diagram of the construction of plasmid pGLHRE (Fig. 1B) from plasmid pGL2PV (Fig. 1A).

Figures 2A, 2B, 2C and 2D show a schematic diagram of the construction of plasmids pGLHSA-150HRE (Fig. 2B), pGLα$MHC_{86}$-HRE (Fig. 2C), and $pGLHCA_{118}$HRE (Fig. 2D), from plasmid pGLHRE (Fig. 2A).

Figures 3A and 3B show a schematic diagram of the construction of plasmid pGLα$MHC_{1.2}$HRE (Fig. 3B) from plasmid pGLHRE (Fig. 3A).

Figures 4A and 4B show a schematic diagram of the construction of plasmid pGLα$MHC_{1.2}$HRE-NOS (Fig. 4B) from plasmid pGLα$MHC_{1.2}$HRE (Fig. 4A).

Figures 5A and 5B show a schematic diagram of the construction of plasmid p$\alpha$MHC$_{1.2}$-HRE-Bcl-2 (Fig. 5B) from plasmid pSFFV-Bcl-2 (Fig. 5A).

Figures 6A, 6B, 6C, 6D and 6E show a schematic diagram of the construction of plasmids pGLPKM$_{460}$ (Fig. 6C), pGLPKM$_{D}$ (Fig. 6D), and pGLPKM$_{285}$ (Fig. 6E) from plasmid pGL2BV (Fig. 6B) and a fragment of the PKM promoter (Fig. 6A; SEQ ID NO:7).

Figures 7A, 7B and 7C show a schematic diagram of the construction of plasmid pGLET-1$_{700}$ (Fig. 7C) from plasmid pGL2BV (Fig. 7B) and a fragment of the ET-1 promoter (Fig. 7A; SEQ ID NO:8).

## Brief Description of the Sequences

SEQ ID NO:1 is the sense strand nucleotide sequence of a GATA4 enhancer element (Molkentin, *et al.*, 1984).

SEQ ID NO:2 is the nucleotide sequence of muscle pyruvate kinase (PKM) sense strand primer F.

SEQ ID NO:3 is the nucleotide sequence of PKM reverse strand primer R.

SEQ ID NO:4 is the nucleotide sequence of endothelin-1 (Et-1) sense strand primer F.

SEQ ID NO:5 is the nucleotide sequence of Et-1 reverse strand primer R.

SEQ ID NO:6 is the nucleotide sequence of hypoxia response enhancer element 1 (HREE1), derived from the erythropoietin (EPO) gene (Semenza and Wang), and containing 4 tandem copies of a hypoxia response enhancer (HRE) sequence and cloning linkers.

SEQ ID NO:7 is the nucleotide sequence of a rat muscle pyruvate kinase (PKM) promoter region (Takenaka, *et al.*).

SEQ ID NO:8 is the nucleotide sequence of a human Et-1 promoter region (Inoue, *et al.*).

SEQ ID NO:9 is the nucleotide sequence of a human cardiac actin promoter region (Minty and Kedes).

SEQ ID NO:10 is a nucleotide sequence containing a portion of the rat cardiac $\alpha$-myosin heavy chain promoter region (Mahdavi, *et al.*; GenBank Accession # K01464).

SEQ ID NO:11 is a nucleotide sequence containing a portion of the mouse cardiac $\alpha$-myosin heavy chain promoter region (Gulick, J., *et al.*; GenBank Accession # M62404).

SEQ ID NO:12 is the nucleotide sequence of a human B-cell leukemia/lymphoma 2 (bcl-2) gene (Tsujimoto, *et al.*; GenBank Accession # M13994).

SEQ ID NO:13 is the predicted amino acid sequence from SEQ ID NO:12.

SEQ ID NO:14 is the nucleotide sequence of a rat nitric oxide synthase (bNOS) gene (Bredt, *et al.*; EMBL Accession # X59949).

SEQ ID NO:15 is the predicted amino acid sequence from SEQ ID NO:14.

SEQ ID NO:16 is the nucleotide sequence of a human bcl-2 fusion gene (Seto, *et al.*; EMBL Accession # X06487).

SEQ ID NO:17 is the predicted amino acid sequence from SEQ ID NO:16.

SEQ ID NO:18 is the nucleotide sequence of a human NOS-1 gene (Fujisawa, *et al.*); DDBJ Accession # D16408; NCBI Seq ID 506339)

SEQ ID NO:19 is the predicted amino acid sequence from SEQ ID NO:18.

SEQ ID NO:20 is the nucleotide sequence of a human NOS-SN gene (Nakane, *et al.*; GenBank Accession # L02881)

SEQ ID NO:21 is the predicted amino acid sequence from SEQ ID NO:20.

SEQ ID NO:22 is the nucleotide sequence of a 256 base pair (bp) 3' EPO-1 hypoxia response enhancer element (Semenza and Wang).

SEQ ID NO:23 is the nucleotide sequence of a 42 bp 3' EPO-1 hypoxia response enhancer element (Madan, *et al.*).

SEQ ID NO:24 is the nucleotide sequence of an 86 bp rat αMHC promoter region.

SEQ ID NO:25 is the nucleotide sequence of a mouse catalase gene (Reimer, *et al.*; GenBank #L25069).

SEQ ID NO:26 is the predicted amino acid sequence from SEQ ID NO:25.

SEQ ID NO:27 is the nucleotide sequence of a human manganese superoxide dismutase (SOD) gene (Clair, *et al.*; EMBL #X59445).

SEQ ID NO:28 is the predicted amino acid sequence from SEQ ID NO:27.

SEQ ID NO:29 is the nucleotide sequence of a human $\beta$-enolase (ENO3) gene (Giallongo, *et al.*; EMBL #X56832) between nucleotides -628 to +63.

SEQ ID NO:30 is the predicted amino acid sequence from SEQ ID NO:29.

SEQ ID NO:31 is a consensus sequence of a region present in both the PKM and ENO3 promoters.

SEQ ID NO:32 is the DNA sequence of the -760 fragment of the human metallothionein IIA (hMTAIIa) promoter.

SEQ ID NO:33 is the DNA sequence of the -345 fragment of the hMTAIIa promoter.

SEQ ID NO:34 is the DNA sequence of the -163 fragment of the hMTAIIa promoter.

SEQ ID NO:35 is the DNA sequence of the -90 fragment of the hMTAIIa promoter.

SEQ ID NO:36 is a cDNA sequence encoding human tumor necrosis factor (hTNF; EMBL Accession #X01394; Pennica, *et al.*, Shirai, *et al.*).

SEQ ID NO:37 is the predicted amino acid sequence from SEQ ID NO:36.

## DETAILED DESCRIPTION OF THE INVENTION

### Definitions

"Ischemia" is defined as an insufficient supply of blood to a specific organ or tissue. A consequence of decreased blood supply is an inadequate supply of oxygen to the organ or tissue (hypoxia). Prolonged hypoxia may result in injury to the affected organ or tissue. "Anoxia" refers to a virtually complete absence of oxygen in the organ or tissue, which, if prolonged, may result in death of the organ or tissue.

"Hypoxic condition" is defined as a condition under which a particular organ or tissue receives an inadequate supply of oxygen.

"Anoxic condition" refers to a condition under which the supply of oxygen to a particular organ or tissue is cut off.

"Reperfusion" refers to the resumption of blood flow in a tissue following a period of ischemia.

"Ischemic injury" refers to cellular and/or molecular damage to an organ or tissue as a result of a period of ischemia and/or ischemia followed by reperfusion.

An "element", when used in the context of nucleic acid constructs, refers to a region of the construct or a nucleic acid fragment having a defined function. For example, a hypoxia response enhancer element is a region of DNA that, when associated with a gene operably linked to a promoter, enhances the transcription of that gene under hypoxic conditions.

The term "operably linked", as used herein, denotes a relationship between a regulatory region (typically a promoter element, but may include an enhancer element) and the coding region of a gene, whereby the transcription of the coding region is under the control of the regulatory region.

Two nucleic acid elements are said to be "heterologous" if the elements are derived from two different genes, or alternatively, two different species. For example, a hypoxia response enhancer element from a human erythropoietin gene is heterologous to a promoter from a human myosin gene. Similarly, a hypoxia response enhancer element from a human erythropoietin gene, for example, is heterologous to a promoter from a mouse erythropoietin gene.

"Control region" refers to specific sequences at the 5' and 3' ends of eukaryotic genes which may be involved in the control of either transcription or translation. For example, most eukaryotic genes have an AT-rich region located approximately 25 to 30 bases upstream from the site where transcription initiation site. Similarly, most eukaryotic genes have a CXCAAT region (X may be any nucleotide) 70 to 80 bases upstream from the start of transcription. At the 3' end of most eukaryotic genes is an AATAAA sequence, which may be the signal for addition of the polyadenylation tail to the 3' end of the transcribed mRNA.

"Chimeric gene" refers to a polynucleotide containing heterologous DNA sequences, such as promoter and enhancer elements operably linked to a therapeutic gene. For example, a construct containing a human $\alpha$-myosin heavy chain ($\alpha$-MHC) promoter fragment operably linked to a human bcl-2 gene and containing a human erythropoietin gene hypoxia response element comprises an exemplary chimeric gene.

## I. Overview of the Invention

The present invention relates to chimeric genes having at least three functional elements: (i) a therapeutic gene, (ii) a tissue-specific promoter, and (iii) a hypoxia response enhancer (HRE) element. The tissue-specific promoter in combination with the HRE element directs expression of the therapeutic gene in a selected tissue under hypoxic conditions.

The gene is preferably introduced into a target tissue as part of a complete expression vector in a pharmaceutically-acceptable vehicle, either by direct administration to the target tissue (*e.g.*, injection into the target tissue), or by systemic administration (*e.g.*, intravenous injection). In the latter case, the gene may be targeted to a selected tissue, for example, by incorporating it in a virion expressing a modified envelope protein designed to bind to receptors preferentially expressed on cells from the selected, or targeted, tissue. Regardless of the delivery means, expression of the gene in tissues other than the target tissue, and under conditions other than hypoxic or anoxic is preferably minimal.

As described below, a variety of therapeutic genes, promoters, HRE elements and gene delivery means may be employed in the practice of the present invention.

## II. Tissue Specific Promoters

A promoter, in the context of the present specification, refers to a polynucleotide element capable of regulating the transcription of a gene adjacent and downstream (3') of the promoter. The promoter may contain all of, or only a portion of, the complete 5' regulatory

sequences of the gene from which it is derived. A sequence in the promoter region is typically recognized by RNA polymerase molecules that start RNA synthesis.

A promoter may be functional in a variety of tissue types and in several different species of organisms, or its function may be restricted to a particular species and/or a particular tissue. Further, a promoter may be constitutively active, or it may be selectively activated by certain substances (*e.g.*, a tissue-specific factor), under certain conditions (*e.g.*, hypoxia, or the presence of an enhancer element in the chimeric gene containing the promoter), or during certain developmental stages of the organism (*e.g.*, active in fetus, silent in adult).

Promoters useful in the practice of the present invention are preferably tissue-specific -- that is, they are capable of driving transcription of a gene in one tissue while remaining largely "silent" in other tissue types. It will be understood, however, that tissue-specific promoters may have a detectable amount of "background" or "base" activity in those tissues where they are silent. The degree to which a promoter is selectively activated in a target tissue can be expressed as a selectivity ratio (activity in a target tissue/activity in a control tissue). In this regard, a tissue specific promoter useful in the practice of the present invention typically has a selectivity ratio of greater than about 5. Preferably, the selectivity ratio is greater than about 15.

It will be further understood that certain promoters, while not restricted in activity to a single tissue type, may nevertheless show selectivity in that they may be active in one group of tissues, and less active or silent in another group. Such promoters are also termed "tissue specific", and are contemplated for use with the present invention. For example, promoters that are active in a variety of central nervous system (CNS) neurons may be therapeutically useful in protecting against damage due to stroke, which may effect any of a number of different regions of the brain.

Tissue-specific promoters may be derived, for example, from promoter regions of genes that are differentially expressed in different tissues. For example, a variety of promoters have been identified which are suitable for upregulating expression in cardiac tissue. Included are the cardiac $\alpha$-myosin heavy chain ($\alpha$MHC) promoter and the cardiac $\alpha$-actin promoter.

A further desirable characteristic of promoters useful in the present invention is that they possess a relatively low activity in the absence of activated hypoxia-regulated enhancer elements, even in the target tissues. One means of achieving this is to select promoters of genes encoding proteins that have a relatively low turnover rate in adult tissue, such as the actin and $\alpha$-MHC promoters described herein. Another means is to use "silencer" elements, which suppress the activity of a selected promoter in the absence of hypoxia.

The level of expression of a gene under the control of a particular promoter can be modulated by manipulating the promoter region. For example, different domains within a promoter region may possess different gene-regulatory activities. The roles of these different regions are typically assessed using vector constructs having different variants of the promoter with specific regions deleted (*i.e.*, deletion analysis). Vectors used for such experiments typically contains a reporter gene, which is used to determine the activity of each promoter variant under different conditions. Application of such a deletion analysis enables the identification of promoter sequences containing desirable activities.

This approach may be used to identify, for example, the smallest region capable of conferring tissue specificity, or the smallest region conferring hypoxia sensitivity.

A number of tissue specific promoters, described below, may be particularly advantageous in practicing the present invention. In most instances, these promoters may be isolated as convenient restriction digest fragments suitable for cloning into a selected vector.

Alternatively, promoter fragments may be isolated using the polymerase chain reaction (PCR; Mullis, Mullis, *et al.*). Cloning of amplified fragments may be facilitated by incorporating restriction sites at the 5' ends of the primers.

Promoters suitable for cardiac-specific expression include the promoter from the murine cardiac $\alpha$-myosin heavy chain gene. The gene contains a 5.5 kbp promoter region which may be obtained as a 5.5 kbp *SacI/SalI* fragment from the murine $\alpha$MHC gene (Subramaniam, *et al.*, 1991). Reporter gene constructs utilizing this 5.5 kbp $\alpha$MHC promoter are expressed at relatively high levels selectively in cardiac tissue (whether or not an HREE is present) and, when present in transgenic animals, are regulated in a similar fashion to the endogenous gene (Subramaniam, *et al.*, 1991).

A smaller fragment of the rat $\alpha$-MHC promoter may be obtained as a 1.2 kbp *EcoRI/Hind*III fragment (Gustafson, *et al.*). As shown in Example 1 and Table 1, below, constructs utilizing the 1.2 kbp rat $\alpha$MHC promoter are expressed at a low level in the absence of an HREE, and at an intermediate level in the presence of an HREE. These results indicate that the $\alpha MHC_{1.2}$ promoter is an exemplary promoter to target expression of chimeric genes of the present invention to cardiac tissue. Expression of genes under the control of this promoter fragment is very low in cardiac cells under normal oxygenation conditions, but is increased by about a factor of four under hypoxic conditions when the construct contains HREE1. Expression in cells other than cardiac cells is at background levels.

An 86 bp fragment of the rat $\alpha$MHC promoter, presented herein as SEQ ID NO:24, restricts expression of reporter genes to cardiac and skeletal muscle (*i.e.*, it has lost some tissue

selectivity). Additional cardiac specificity may be conferred to the fragment by ligating (*e.g.*, blunt end ligating) a 36-mer oligonucleotide (SEQ ID NO:1) containing cardiac-specific GATA4 enhancer elements just upstream of base pair -86 (Molkentin, *et al.*, 1984). This promoter fragment also results in low levels of expression in the absence of additional enhancers such as HRE elements. The low level of basal expression induced by the 86 bp fragment, and the ability to upregulate this basal level of expression with a hypoxia response enhancer element are useful properties for a promoter for use with the present invention.

The sequences of exemplary cardiac-specific promoter regions from the rat and mouse αMHC genes are presented herein as SEQ ID NO:10 and SEQ ID NO:11, respectively. Both sequences end just upstream of the ATG initiation codons of their respective genes. Other cardiac-specific promoters include the cardiac α-actin promoter and the myosin light chain-2 (MLC-2) promoter. Constructs described herein utilizing a 118 bp fragment (SEQ ID NO:9) from the human cardiac α-actin (HCA) promoter result in a relatively low level of cardiac-specific expression, which may be increased by the inclusion of an HREE in the expression construct (Example 1, Table 1). The cardiac-specific myosin light chain-2 promoter may be obtained as a 2.1 kbp *KpnI/EcoRI* fragment from the rat cardiac myosin light chain-2 (MLC-2) gene (Franz, *et al.*).

Prostate-specific promoters include the 5'-flanking regions of the human glandular kallikrein-1 (hKLK2) gene and the prostate-specific antigen (hKLK3; PSA) gene (Murtha, *et al.*; Luke, *et al.*). The renin promoter is suitable for directing kidney-specific expression (Fukamizu, *et al.*), while the aldolase-C promoter (Vibert, *et al.*) or the tyrosine hydroxylase promoter (Sasaoka, *et al.*) may be used to direct expression in the brain. Promoters specific for vascular endothelium cells include the Et-1 promoter (Inoue, *et al.*) and vonWillebrand factor (Jahrondi and Lynch) promoter.

Tumor-specific promoters include the α-fetoprotein (AFP) promoter, contained in a 7.6 kbp fragment of 5'-flanking DNA from the mouse AFP gene (Marci, *et al.*). This promoter normally directs expression of the AFP gene in fetal liver and is transcriptionally silent in adult tissues. However, it can be abnormally reactivated in hepatocellular carcinoma (HCC), conferring tumor-specific expression in adult tissue (Marci, *et al.*).

The above promoters are exemplary promoters for use with the present invention. Other promoters suitable for use with the present invention may be selected by one of ordinary skill in the art following the guidance of the present specification.

III. Hypoxia Response Enhancer Elements

Therapeutic genes contained in constructs of the present invention are preferably expressed at low levels, if at all, under conditions of normal oxygenation (minimizing any side effects). Under conditions of hypoxia, however, expression of the genes is increased, affording protection to the target tissue. The elevated expression under hypoxic conditions is conferred by the presence of one or more hypoxia response enhancer (HRE) elements.

HRE elements contain polynucleotide sequences that may be located either upstream (5') or downstream (3') of the promoter and/or therapeutic gene. The HRE element (HREE) is typically a *cis*-acting element, usually about 10-300 bp in length, that acts on a promoter to increase the transcription of a gene under the control of the promoter. Preferably, the promoter and enhancer elements are selected such that expression of a gene regulated by those elements is minimal in the presence of a healthy supply of oxygen, and is upregulated under hypoxic or anoxic conditions.

Hypoxia response enhancer elements are found in association with a number of genes, including the erythropoietin (EPO) gene. Exemplary HRE elements from the EPO gene are presented herein as SEQ ID NO:6, SEQ ID NO:22 and SEQ ID NO:23. The element having the sequence represented as SEQ ID NO:22 results in approximately a five-fold induction of reporter gene expression under hypoxic conditions (Semenza and Wang), while, the element having the sequence represented as SEQ ID NO:23 results in approximately a 17-fold increase in activity under hypoxic conditions (Madan, *et al.*)

Experiments performed in support of the present invention (*e.g.*, Example 1) demonstrate that expression of constructs containing HREE1 (SEQ ID NO:6) is increased by approximately 5- to 7-fold in response to hypoxic conditions. These results indicate that the HREE1 element is fully functional when fused to muscle and cardiac specific promoters and that muscle and cardiac cells are fully responsive to hypoxia in terms of the regulation of these promoters.

Expression of constructs containing a fragment (SEQ ID NO:29) from the control region of the enolase 3 (ENO3) gene was induced approximately 5 to 8 fold by hypoxia in C2C12 cells and cardiac myocytes respectively (see Table 1). These results suggest that the HREE in the ENO3 promoter fragment may be a particularly effective HREE for hypoxia induction in constructs containing a tissue-specific promoter, such as a cardiac or skeletal muscle promoter.

According to the present invention, exemplary hypoxia response enhancer elements may also be isolated from regulatory regions of both the muscle glycolytic enzyme pyruvate kinase

(PKM) gene (Takenaka, *et al.*), the human muscle-specific $\beta$-enolase gene (ENO3; Peshavaria and Day), and the endothelin-1 (ET-1) gene (Inoue, *et al.*). The HRE regions from the PKM gene and the ET-1 gene, identified in experiments performed in support of the present invention (see Materials and Methods, Examples 4 and 5), are presented herein as SEQ ID NO:7 and SEQ ID NO:8, respectively.

Example 4 demonstrates that the expression of pGLPKM, a plasmid containing the HRE element from the PKM gene, in transfected C2C12 myotubes and neonatal cardiac myocytes was increased by $6 \pm 2$ ($n = 4$) fold in both cell types by incubation of the cells in a hypoxic atmosphere. A portion of this HRE element, obtained by digesting with *Sma*I to cut at an internal *Sma*I site, localized the hypoxia response sequence to a 200 bp fragment. This fragment, termed $\mathrm{HREPKM}_{285}$, confers hypoxia-induced expression in C2C12 myotubes and cardiac myocytes that is at least equivalent to that obtained using HREE1 (SEQ ID NO:6).

Both PKM and ENO3 promoters contain a common sequence element (SEQ ID NO:31) located at 5' -88 and -70 bp respectively from the transcription start sites. An oligonucleotide containing this sequence may be sufficient to confer hypoxia response characteristics to constructs of the present invention.

Data presented in Example 5 show that expression of pGLET-$1_{700}$, containing 700 bp of the human ET-1 gene promoter (SEQ ID NO:8), in transfected human arterial endothelial cells was increased approximately 5 -fold by incubation of the cells in a hypoxic atmosphere. No hypoxia-induced increase in pGLET-$1_{700}$ expression was seen in other cell types, including HeLa cells, C2C12 cells, and cardiac myocytes. Accordingly, the 700 bp fragment may be used to target hypoxia regulated genes specifically to cells of the vascular endothelium, since the fragment contains element(s) conferring tissue specificity (i.e., elements effective to target expression exclusively to the vascular endothelium), as well as HRE element(s) effective to upregulate transcription of a gene under control of the fragment during hypoxic conditions.

Data presented in Example 6 show that hypoxic stress can increase transcription from constructs containing fragments of the hMTIIa proximal promoter. Enhancements in CAT activity relative to the aerobic controls were observed at both 8 and 14 hr of hypoxia. The levels of induction (2-3 fold) were within the same range as those found in the cadmium chloride-treated positive controls. Hypoxia responsiveness of the -760 construct (SEQ ID NO:32) was similar to that of the -345 (SEQ ID NO:33) construct.

Deletion analyses described in Example 7 show that extracts from cells transfected with constructs containing the -163 fragment (SEQ ID NO:34) and the -90 fragment (SEQ ID NO:35) showed significant upregulation of reporter activity (luciferase activity) under hypoxic

conditions, with levels of induction (approximately 3.0-fold) similar to those observed in Example 6. These results suggest that at least one HRE element is contained in the proximal 90 bp fragment (SEQ ID NO:35) of the hMTIIa promoter. Such an HRE element may be utilized in the methods and constructs of the present invention.

It will be appreciated that deletion analyses such as described in Example 7 may be used to identify the shortest sequence present in the -90 fragment (SEQ ID NO:35) that still confers hypoxia sensitivity or inducibility, and that this shorter sequence may be used as the HRE element in the compositions and methods of the present invention.

It will further be appreciated that the present invention includes the use of HRE elements not explicitly identified above. Additional HRE elements may be identified, for example, as detailed in Examples 4 and 5. Further, promoter deletion and mutation analyses (*e.g.*, as described above and in Webster and Kedes) may be used to identify such elements in other hypoxia responsive genes. A number of such responsive target genes have been shown to be induced when cells are exposed to hypoxia *in vitro* (*e.g.*, Heakock and Sutherland).

It will also be appreciated that, in certain circumstances, the tissue-specific promoter and the hypoxia response enhancer element(s) of the present invention may be derived from a contiguous polynucleotide sequence from a single gene (*e.g.*, as shown above for the ET-1 promoter region, which contains HRE element(s) and also imparts endothelial cell-specific expression).

## IV. Therapeutic Genes

The present invention may be used to alleviate a number of disease conditions resulting from hypoxic and/or anoxic conditions due to ischemia where cell and tissue damage results from ischemia and ischemia followed by reperfusion. The invention is particularly suitable in cases where the subject is diagnosed to be at risk for an ischemic episode in a particular tissue.

For example, it is recognized that virtually all surviving heart attack victims are at significantly increased risk for recurrent episodes of myocardial ischemia. Such subjects would benefit from the introduction of constructs capable of expressing therapeutic genes into their cardiac tissue in order to decrease the risk of injury to the tissue during any subsequent ischemic episodes. Such constructs may serve to protect, for example, cardiac and vascular endothelial tissues from ischemic damage and thereby prevent the progression of the heart disease.

Recurrent ischemia and reperfusion typically results in oxidative damage to cells from reactive oxygen species (free radicals), such as peroxides, that are generated during redox

switching (Frei). Contact of fresh blood with damaged or dead cells induces the influx of neutrophils, or pus cells, which kill heart cells which would otherwise have recovered. Much of the damage caused by neutrophils has been attributed to superoxide ions. The superoxide anion can damage tissue in several ways. The interaction of the superoxide anion with hydrogen peroxide leads to the production of hydroxyl radicals which are potentially toxic and react rapidly with most organic molecules. Lipids, proteins, and nucleic acids may all be primary targets for such oxidative damage. The extent and type of damage depend on the severity and nature of the hypoxic stress. For example, the stress may cause cellular damage, initiating an inflammatory response with neutrophil attack and subsequent tissue necrosis. Alternatively, the stress may initiate apoptosis (programmed cell death) to eliminate the damaged cells.

Regardless of the mechanism by which tissue death occurs (necrosis or apoptosis), the damage caused by ischemia-reperfusion episodes is typically the result of redox reactions and is quantitatively related to the severity and duration of the ischemia. For example, in the case of the myocardium, a severe heart attack may result in extensive damage (*e.g.*, infarction of 30% to 40% of the left ventricle), whereas moderate angina and silent repetitive ischemia may result in relatively minor damage during each episode.

While the pathology of ischemia in tissues is complex, resulting in multiple potential targets for therapeutic intervention, several classes of targets are particularly suitable for therapeutic intervention in accordance with the teachings of the present invention. These include anti-oxidant systems, that may intervene immediately at the sites of intracellular redox reactions to minimize damage, and vasodilator systems, that may minimize the severity of the ischemia by increasing blood flow to vulnerable tissues. Antioxidant proteins amenable for use with the present invention include gene products of Bcl-2, catalase and superoxide dismutase (SOD) genes, while proteins with vasodilative properties include nitric oxide synthase (NOS), which produces the vasodilator nitric oxide (NO).

Bcl-2, an integral inner mitochondrial membrane protein of relative molecular mass ~25 kDa, has been shown to protect certain cells against apoptosis (Hockenbery, *et al.*, 1990) by acting as an antioxidant (Hockenbery, *et al.*, 1993). Bcl-2 may be an effective therapeutic gene for reducing damage to tissues during ischemic episodes because apoptosis may be a common response of many tissues, including the heart, to oxidative stress (Williams and Smith; Gottlieb, *et al.*

The enzyme superoxide dismutase (SOD) catalyzes the decomposition of the superoxide anion to peroxide. Enzymes such as superoxide dismutase, free radical scavengers or agents

which prevent the influx on neutrophils are able to increase the salvage of heart muscle cells. The enzyme catalase in turn catalyzes the conversion of peroxides to water. Exemplary sequences of a SOD gene and a catalase gene are presented herein as SEQ ID NO:27 and SEQ ID NO:25, respectively. The sequence presented herein as SEQ ID NO:27 encodes a manganese SOD, which has a relatively long half-life. A related sequence, of a human Cu/Zn SOD, may be found in Gorechi, *et al.* The Cu/Zn SOD has a shorter half-life than the manganese SOD.

Endothelial-derived nitric oxide (NO) regulates the expression of vasoconstrictors and growth factors by the vascular endothelium (Kourembanas, *et al.*). Under hypoxia, endothelial cells typically increase expression and secretion of endothelin-1 (ET-1), a potent vasoconstrictor. This increase in expression can be reduced or prevented by exposure to NO (Kourembanas, *et al.*). One of the effects of ET-1 induced vasoconstriction is decreased blood flow to the affected organ or tissue, which can exasperate hypoxic damage due to ischemia. According to the present invention, such damage may be reduced by providing NO to the affected tissue through the expression of a NOS gene under the control of a vascular epithelium or cardiac-specific promoter and hypoxia response enhancer element.

Therapeutic genes of the present invention may be preferably derived from the same or related species as the one to which the methods and compositions of the present invention are applied. For example, for therapeutic treatment of a dog, it may be desirable to utilize a construct containing a therapeutic gene cloned from a dog. Similarly, for treatment of human conditions, it may be desirable to utilize therapeutic genes cloned from human-derived nucleic acids.

The genes encoding the proteins discussed above represent exemplary therapeutic genes useful in the practice of the present invention. It will be appreciated, however, that following the teachings and guidance of the present specification, one of skill in the art may select other therapeutic genes effective to reduce cellular damage due to hypoxia or ischemia, and that the use of such genes is considered to be within the scope of the present invention.

## V. Deleterious Genes

In another aspect, the present invention includes constructs containing deleterious genes, rather than therapeutic genes. Expression of the deleterious genes is targeted to tissues which are harmful (*e.g.*, malignant tumors) or otherwise undesirable. Promoters and hypoxia response elements may be selected as described above. Promoters useful in this aspect of the invention preferably restrict expression only to the undesirable tissue. For example, as

discussed above, the AFP promoter can be activated in hepatocellular carcinoma (HCC), conferring tumor-specific expression in adult tissues (Marci, *et al.*).

Deleterious genes include a viral thymidine kinase gene (tk), such as the herpes simplex virus (HSV) tk. This gene is not deleterious by itself, but when expressed, viral TK can phosphorylate ganciclovir (GCV), turning GCV into a cytotoxic compound. Since tumor cells are typically hypoxic, constructs having a tumor-specific promoter operably linked to a viral tk and an HREE may be used in conjunction with GCV to selectively kill tumor cells. Another exemplary deleterious gene is tumor necrosis factor (TNF). TNF is a growth factor that rapidly and induces programmed cell death or apoptosis (Cleveland and Ihle, 1995).

## VI. Expression Vectors

Chimeric genes of the present invention are preferably incorporated into expression vectors capable of expressing a therapeutic gene product in a selected eukaryotic host cell (*i.e.*, a target tissue). Such expression vectors may contain, in addition to the chimeric gene, various other sequences useful for effective expression of the therapeutic gene in selected tissues. Such sequences may include, for example, sequences necessary for the termination of transcription. These sequences are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding the desired therapeutic protein. The 3' untranslated regions may also include transcription termination sites.

Molecular techniques and methods useful in the construction of expression vectors are well known in the art (*e.g.*, Ausubel, *et al.*, Sambrook, *et al.*). Vector constructs made in support of the present invention are designed to express either a reporter gene (*e.g.*, luciferase), or therapeutic genes (*e.g.*, Bcl-2 or NOS). Therapeutic gene expression is under the control of either a ubiquitous promoter (*e.g.*, SV40), or a tissue-specific promoter (e.g., striated muscle or cardiac-specific promoter). Further regulation of expression by hypoxia or anoxia is provided by inclusion of hypoxia response enhancer (HRE) elements (*e.g.*, from the erythropoietin (EPO) gene, muscle specific pyruvate kinase (PKM) gene, enolase 3 (ENO3) gene or the endothelial cell endothelin-1 (Et-1) gene).

The generation of exemplary constructs is described in the Materials and Methods section, below. The results of *in vitro* experiments to assess the performance of constructs having HREE1 and tissue specific promoters are presented in Example 1 and Table 1. The relative amount of gene expression was measured using a reporter gene (luciferase) in place of a therapeutic gene.

The data shown in Table 1 demonstrate that cells containing constructs having a hypoxia response enhancer element, such as HREE1, in combination with a compatible promoter, express the reporter at levels that are 5 to 7 times greater under hypoxic conditions than under aerobic conditions, and that HREE1 is equally active in different cells and independent of the promoter. The data also demonstrate that expression of constructs containing $\alpha$-MHC promoters is cardiac specific, and that the basal (aerobic) expression from $\alpha$-$MHC_{1.2}$ and HCA promoters is relatively low. Further, the data indicate that muscle and cardiac cells are fully responsive to hypoxia in terms of the regulation of these promoters.

*In vivo* experiments conducted with plasmids pGLHRE and $pGLHCA_{118}HRE$ (Example 2, Table 2) demonstrate that gene expression in hearts of rats injected with the plasmids and subjected to ischemia was approximately 2-fold higher than expression in hearts from control animals (not subjected to ischemia). These results indicate that the direct injection of therapeutic constructs of the present invention into cardiac tissue *in vivo* is effective to result in the expression of genes carried on those plasmids. Further, these results indicate that expression vectors carrying chimeric genes of the present invention are effective to result in significantly increased levels of expression in response to hypoxia caused by ischemia *in vivo*.

Since expression was measured at 20 hours after a brief (20 minute) episode of ischemia, it will be appreciated that (i) hypoxia-induced expression may peak significantly earlier than 20 hours, and (ii) repeat ischemic episodes may upregulate expression more the single experimental episode used herein. Accordingly, the 2-fold induction may be an underestimate of the level of enhancement of transcription/expression caused by ischemia.

While the experiments described above were performed with cardiac tissue, it will be appreciated that one of ordinary skill in the art having the benefit of the present specification may perform similar manipulations with other tissues subject to ischemic and or ischemic/reperfusion injury, and that such procedures are within the scope of the present invention.

*In vitro* experiments (Example 3) demonstrate that cells transfected with reporter (pGLHRE, $pGLHCA_{118}HRE$, $pGL\alpha MHC_{1.2}HRE$) and therapeutic (pSFFV-Bcl-2 and pNOS-HRE) constructs appear normal and respond to stimuli as expected. Reporter-transfected cells differentiate normally and respond to hypoxia with the predicted induction of reporter, while NOS and bcl-2-transfected cells appear normal both during the hypoxia and during subsequent reoxygenation. These results suggest that inclusion of HRE elements, Bcl-2 over-expression, and hypoxia-induced over-expression of NOS is not toxic or deleterious to muscle cells *in vitro*.

These results also suggest that expression vectors carrying therapeutic genes of the present invention may be effective to protect tissues from ischemic damage. Such protective effects may be assayed in an animal model by, for example, infecting myocardial tissue with an expression vector containing a chimeric gene of the present invention, such as an adenoviral vector expressing a therapeutic gene (*e.g.*, Bcl-2 or SOD), a cardiac-specific promoter, and an HRE element, as described, for instance, in Example 2.

Following infection, the animals may be subjected to repeat ischemic episodes (*e.g.*, 30 minutes to 1 hour) followed by reperfusion (*e.g.*, 1 to 8 hours). Following the last reperfusion, the animals may be sacrificed and the ischemic regions of the myocardium may be tested for the presence and extent of infarction as described, for example, by Thornton, *et al.*, and for the presence of apoptosis as described, for example, in Gottlieb, *et al.* Sample biopsies may also be assayed for expression of the therapeutic gene by Northern blots.

Similar experiments may be performed using constructs directed (*e.g.*, via an appropriate promoter) to other tissues, such as brain, kidney and vascular endothelium.

Examples 8 and 9 describe exemplary constructs containing an HRE element from the hMTIIa promoter and a deleterious gene (TNF). The examples describe the testing of such constructs both *in vitro* (Example 8) and *in vivo* (Example 9).

VII. Delivery of Constructs to Cells and Tissues

Any of a variety of methods known to those skilled in the art may be used to introduce chimeric genes of the present invention into selected target tissue cells. For example, gene therapy of cardiac tissue has included lipofection, retrovirus and adenovirus-mediated gene transfer, and injection of naked DNA directly into the vascular endothelium or cardiac tissue (Nabel, et al.; Lin, *et al.*; Leclere, *et al.*; Flugelman, *et al.*). These and other methods are discussed more fully in the sections below.

Viral-Mediated Gene Transfer.

Host cells may be transfected with chimeric genes of the present invention by infection with mature virions containing hybrid vectors (the chimeric genes along with selected viral sequences). The virions used to transfect host cells are preferably replication-defective, such that the virus is not able to replicate in the host cells.

The virions may be produced by co-infection of cultured host cells with a helper virus. Following coinfection, the virions are isolated (*e.g.*, by cesium chloride centrifugation) and any remaining helper virus is inactivated (*e.g.*, by heating). The resulting mature virions contain

a chimeric gene of the present invention and may be used to infect host cells in the absence of helper virus. Alternatively, high titers of replication-defective recombinant virus, free f helper virus, may be produced in packaging cell lines containing those components for which the virus is defective (Miller).

Several types of viruses, including retroviruses, adeno-associated virus (AAV), herpes virus, vaccinia virus, and several RNA viruses may be amenable for use as vectors with chimeric gene constructs of the present invention. Each type of virus has specific advantages and disadvantages, which are appreciated by those of skill in the art. Methods for manipulating viral vectors are also known in the art (*e.g.*, Grunhaus and Horowitz; Hertz and Gerard; and Rosenfeld, *et al.*)

Retroviruses, like adeno-associated viruses, stably integrate their DNA into the chromosomal DNA of the target cell. Unlike adeno-associated viruses, however, retroviruses typically require replication of the target cells in order for proviral integration to occur. Accordingly, successful gene transfer with retroviral vectors depends on the ability to at least transiently induce proliferation of the target cells.

Retroviral vectors are attractive in part due to the efficiency of transfection -- some vectors can stably transduce close to 100% of target cells. The use of retroviral vectors for *in vivo* gene therapy has been limited, in part, by the requirement of appropriate viral receptors on the target cell. Because the identities of most retroviral receptors are unknown, it has not been possible to determine the distribution of receptors in different cell types. Accordingly, the targeting of specific cell types by retroviral vectors has in many cases proven problematic.

This difficulty may be circumvented by modifying the envelope protein of the retrovirus to contain a ligand for a known endogenous (not necessarily viral) receptor expressed on the target cells. An application of this technique is described in detail by Kasahara. Preferably, the virus also contains an unmodified envelope protein to facilitate cell entry. A number of receptors, such as desmin, E-selectin, and A-CAM, are expressed preferentially on cardiac cells and may be amenable to this approach (*e.g.*, Hansen and Stawaski; Lefer, *et al.*; Youker, *et al.*).

Adeno-associated viruses are capable of efficiently infecting nondividing cells and expressing large amounts of gene product. Furthermore, the virus particle is relatively stable and amenable to purification and concentration. Replication-defective adenoviruses lacking portions of the E1 region of the viral genome may be propagated by growth in cells engineered to express the E1 genes (Jones and Shenk; Berkner; Graham and Prevea). Most of the currently-used adenovirus vectors carry deletions in the E1A-E1B and E3 regions of the viral

genome. A number of preclinical studies using adenoviral vectors have demonstrated that the vectors are efficient at transforming significant fractions of cells *in vivo*, and that vector-mediated gene expression can persist for significant periods of time (Rosenfeld, *et al.*; Quantin, *et al.*; Stratford-Perricaudet, *et al.*, 1992a; Rosenfeld, *et al.*; L. D. Stratford-Perricaudet, *et al.*, 1992b; Jaffe, *et al.*). Several studies describe the effectiveness of adenovirus-mediated gene transfer to cardiac myocytes (Kass-Eisler, *et al.*; Kirshenbaum, *et al.*).

Herpes virus vectors (Breakefield and DeLuca; Freese, *et al.*) are particularly well suited for the delivery and expression of foreign DNA in cells of the central nervous system (CNS), since they can efficiently infect mature, postmitotic neurons. Methods for manipulating the vectors and transfecting CNS cells are well known (see, *e.g.*, Kennedy and Steiner; Yung). A number of studies describe methods for transplanting genetically modified cells into different regions of the brain (Malim, *et al.*; Rossi and Sarver; Sullenger, *et al.*; Morgan, *et al.*; Chatterjee, *et al.*; Malin, *et al.*; Hope, *et al.*). Studies utilizing direct injection of vectors into CNS tissue have also been performed (*e.g.*, Zhang, *et al.*).

Naked DNA injection

Plasmids bearing chimeric genes of the present invention may be purified and injected directly into a target tissue, as exemplified in Example 2 for rat cardiac tissue. The data discussed in Example 2 demonstrate that cardiac injection of plasmid suspended in saline buffer is effective to result in expression of the plasmid in the cardiac cells. Similar approaches have been used successfully by others to express, for example, exogenous genes in rodent cardiac and skeletal muscle (Wolf, *et al.*; Ascadi, *et al.*, 1991a; Ascadi, *et al.*, 1991b; Lin, *et al.*; Kitsis, *et al.*.

Liposome-Mediated Gene Transfer

Liposomes may be employed to deliver genes to target tissues using methods known in the art. The liposomes may be constructed to contain a targeting moiety or ligand, such as an antigen, an antibody, or a virus on their surface to facilitate delivery to the appropriate tissue. For example, liposomes prepared with ultraviolet (UV) inactivated Hemagglutinating Virus of Japan (HVJ) may be used to deliver DNA to selected tissues (Morishita, *et al.*).

The liposomes may also be surface-coated, *e.g.*, by incorporation of phospholipid - polyethyleneglycol conjugates, to extend blood circulation time and allow for greater targeting via the bloodstream. Liposomes of this type are well known.

Receptor-Mediated Gene Transfer

Receptor-mediated endocytic pathways for the uptake of DNA may permit the targeted delivery of genes to specific cell types *in vivo*. Receptor-mediated methods of gene transfer involve the generation of complexes between plasmid DNA and specific polypeptide ligands (Wu) that can be recognized by receptors on the cell surface. One of the problems with receptor-mediated uptake for gene delivery is that the endocytic vesicles formed during this process may be transported to the lysosome, where the contents of the endosome are degraded. Methods have been developed to facilitate escape of the DNA from the endosome during the course of its transport. For example, either whole adenovirus (Wagner, *et al.*, 1992a; Christiano, *et al.*) or fusogenic peptides of the influenza HA gene product (Wagner, *et al.*, 1992b) may be used to induce efficient disruption of DNA-containing endosomes.

Administration of Constructs

In cases such as those outlined above, where a vector may be targeted to selectively transfect a specific population of cells, it will be understood that in addition to local administration (such as may be achieved by injection into the target tissue), the vector may be administered systemically (e.g., intravenously) in a biologically-compatible solution or pharmaceutically acceptable delivery vehicle. Vector constructs administered in this way may selectively infect the target tissue. According to the present invention, the presence of a target tissue-specific promoter on the construct provides an independent means of restricting expression of the therapeutic gene.

VIII. Applications

A. Therapeutic Applications

Compositions and methods of the present invention may be useful to prevent tissue damage and/or death, due to ischemia and/or subsequent reperfusion, in a variety of tissues. As stated above, an exemplary application is in the reduction of damage due to recurrent myocardial ischemia following a heart attack. The expression of therapeutic genes in the cardiac tissue of heart attack victims may decrease the risk of injury to the tissue during any subsequent ischemic episodes.

Similarly, subjects who have been diagnosed with transient cerebral ischemia, blood clots or other risk factors for stroke may benefit from the use of hypoxia-inducible brain-specific constructs. Subjects diagnosed with acute or chronic renal failure are at greater risk for further ischemic damage to the kidneys (*e.g.*, Rosenberg and Paller). Such subjects may

benefit from a therapeutic gene under the control of a kidney-specific promoter, expression of which is enhanced by hypoxic conditions. A variety of other tissues diagnosed as "at risk" for ischemia may be similarly protected, as will be appreciated by one of skill in the art having the benefit of the present specification.

In addition to the utilities discussed above, compositions (*e.g.*, expression vectors containing chimeric genes of the present invention) and methods of the present invention also have a number of applications in animal medicine. Although animals do not usually develop classical atherosclerosis, cardiomyopathies are very common. A number of species develop ischemia-related syndromes, including arteritis, vasculitis, and related vasculopathies, that result in direct redox damage to cells and tissues, particularly to vascular walls and myocardial tissues. Such conditions may be alleviated by administration of chimeric genes of the present invention.

A common and serious condition in horses and ponies involves ascending colonic ischemia, usually caused by strangulation obstruction (Dabareiner, *et al.*; Sullivan, *et al.*; Wilson and Stick). A related disease in dogs is called gastric dilation-volvulus (Lantz, *et al.*). Treatment of these disorders typically involves surgical removal of the obstruction. Reperfusion following such surgery can result in significant injury to reperfused tissues, and typically triggers an inflammatory response with progressive tissue necrosis. The reperfusion may also results in death of the animal due to cardiogenic shock. Compositions and methods of the present invention may be used therapeutically to treat such conditions, and to provide protection to vulnerable tissues, including heart and vascular endothelium, during the treatment of the above syndromes.

Another utility of the present invention is the treatment of cardiac disease in cats and dogs (Miller, *et al.*). A variety of forms of cardiovascular disease have been described in both cats and dogs, including dilated cardiomyopathy, left ventricular hypertrophy, and hyperthyroidism (Fox, *et al.*; Atkins, *et al.*). Systemic necrotizing vasculitis, a condition that may be analogous to atherosclerosis in humans (with regard to plaque formation and intimal proliferation), has been described in Beagles (Scott-Moncrieff, *et al.*). Each of these conditions may involve ischemia and reperfusion redox injuries to cardiac and vascular tissue that may be treated using the methods and compositions of the present invention.

B. <u>Reporter Constructs for Diagnostic Applications</u>

The present invention may also be employed in diagnostic applications, where it is desirable to localize the site of hypoxia or anoxia. According to this aspect of the invention,

therapeutic genes are replaced by reporter genes, such as those used in experiments performed in support of the present invention (*e.g.*, luciferase). The chimeric genes containing the reporter genes under the control of a selected promoter and a hypoxia response element are introduced into a tissue where it is desirable to localize the site of hypoxia. Hypoxia is localized by increased expression of the reporter gene.

The following examples illustrate but in no way are intended to limit the present invention.

## MATERIALS AND METHODS

Unless indicated otherwise, chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, MO) or Mallinckrodt Specialty Chemicals (Chesterfield, MO), restriction endonucleases were obtained from New England Biolabs (Beverly, MA), and other modifying enzymes and biochemicals were obtained from Pharmacia Biotech (Piscataway, NJ), Boehringer Mannheim (Indianapolis, IN) or Promega Corporation (Madison, WI). Materials for media for cell culture were obtained from Gibco/BRL (Gaithersburg, MD) or DIFCO (Detroit, MI). Unless otherwise indicated, manipulations of cells, bacteria and nucleic acids were performed using standard methods and protocols (*e.g.*, Titus; Sambrook, *et al.*; Ausubel, *et al.*).

### A. Definitions

"Transformation" means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integration. Several transformation methods are commonly used in the art, and may be found, for example, in Ausubel, *et al.*, and Sambrook, *et al.*

"Transfection" refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, $CaPO_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of the expression vector occurs within the host cell.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. "Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences (restriction sites) in the DNA. The

various restriction enzymes used herein are commercially available (*e.g.*, New England Biolabs, Beverly, MA) and their reaction conditions are known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of a plasmid or of a DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 10 μg of DNA are digested with about 20 to 40 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about one hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion, the reaction products are run on a gel (*e.g.*, agarose) to isolate desired fragments.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (*e.g.*, Sambrook, *et al.*). Unless otherwise noted, ligation may be accomplished using known buffers and conditions with 10 units of T4 DNA ligase per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

"Filling" or "blunting" refer to the procedures by which the single stranded end in the cohesive terminus of a restriction enzyme-cleaved nucleic acid is converted to a double strand. This eliminates the cohesive terminus and forms a blunt end. This process is a versatile tool for converting a restriction cut end that may be cohesive with the ends created by only one or a few other restriction enzymes into a terminus compatible with any blunt-cutting restriction endonuclease or other filled cohesive terminus. Typically, blunting is accomplished by incubating 2-15 μg of the target DNA in a buffer containing 10 mM $MgCl_2$, 1 Mm dithiothreitol, 50 mM NaCl, 10 mM Tris (pH 7.5) at about 37°C in the presence of 8 units of the Klenow fragment of DNA polymerase I (Boehringer Mannheim, Indianapolis, IN) and 250 μM of each of the four deoxynucleoside triphosphates (Boehringer Mannheim). The incubation is generally terminated after about 30 min. The reaction products may be purified using standard phenol and chloroform extraction methods followed by ethanol precipitation.

"Northern" blotting is a method by which the presence of a cellular MRNA is confirmed by hybridization to a known, labelled oligonucleotide, DNA or RNA fragment. For the purposes herein, unless otherwise provided, Northern analysis shall mean electrophoretic separation of RNA, typically MRNA, on agarose (*e.g.*, 1%) in the presence of a denaturant (*e.g.*, 7% formaldehyde), transfer to nitrocellulose or nylon membrane, hybridization to the labelled fragment, washing, and detection of the labeled fragment, as described by Sambrook, *et al.*

B. Cells and Media

HeLa cells, Hep G2 cells and C2C12 myoblasts were obtained from the American Type Culture Collection (ATCC; Rockville, MD). Human arterial endothelial cells were obtained from Clonetics Corp. (San Diego, CA). Unless otherwise indicated, the cells were grown at 37°C under 5 or 10% $CO_2$ in MEM or DMEM medium (Gibco/BRL) containing 10% fetal bovine serum (Gibco/BRL).

Cardiac myocytes were isolated and cultured as described previously (Bishopric, *et al.*, Webster and Bisphopric, 1992). Briefly, hearts from about 30 (three litters) were minced and subjected to serial trypsin digestion to release single cells. After the final digestion, the cells were washed and preplated for 0.5 h in minimal essential medium (MEM; Gibco/BRL, Gaithersburg, MD) with 5% fetal calf serum (FCS; Gibco/BRL). Nonattached cells were re-plated in 60-mm Falcon dishes (Becton Dickinson Labware, Lincoln Park, NJ) at a density of about 2.5 × $10^6$ cells per dish in MEM containing 5% fetal calf serum, 2.0 g/l glucose and 10 mM HEPES, and grown at 37°C under 5 or 10% $CO_2$.

C. DNA

1. Therapeutic Genes

Bcl-2 cDNA was obtained in the expression vector pSFFV-Bcl-2 from Dr. Stanley Korsemeyer (Washington University, St. Louis, MO; Hockenbery, *et al.*, 1990). Nitric oxide synthase (bNOS) cDNA was obtained from Dr. Solomon Snyder in the vector pNOS (Johns Hopkins University, Baltimore, MD; Bredt, *et al.*, 1991).

2. Promoters

(i) Cardiac-specific

p$\alpha$MHC$_{5.5}$CAT, containing 5.5 kilobases (Kb) 5' of the mouse $\alpha$-myosin heavy chain ($\alpha$MHC) promoter ligated to the chloramphenicol acetyl transferase (CAT) gene, was obtained from Dr. Jeffrey Robbins (University of Cincinnati, College of Medicine, Cincinnati, Ohio; Subramaniam, *et al.*).

p$\alpha$MHC$_{2.0}$CAT, containing 2.0 Kb of the rat $\alpha$MHC promoter ligated to the CAT gene, was obtained from Dr. Thomas Gustafson (University of Maryland, Baltimore, MD; Gustafson, *et al.*).

p$\alpha$MHC$_{86}$CAT, containing 86 base pairs (bp) of the rat $\alpha$MHC promoter ligated to the CAT gene, was obtained from Dr. Bruce Markham (Medical College of Wisconsin, Milwaukee, Wisconsin). The construct was made by 5' truncation of p$\alpha$MHC2.0CAT and

blunt end ligation to the CAT gene. The sequence of the 86 bp promoter fragment is provided herein as SEQ ID NO:24.

$pHCA_{118}CAT$, containing 118 bp of the region 5' of the human cardiac α-actin promoter ligated to the CAT gene, was also obtained from Dr. Larry Kedes (Minty and Kedes).

(i) Skeletal muscle-specific

pHSA-150CAT, containing 150 bp of the human skeletal muscle α-actin promoter ligated to the CAT gene, was obtained from Dr. Larry Kedes (University of Southern California, Los Angeles, CA; Muscat and Kedes).

3. Hypoxia Response Elements

A construct containing four tandem copies of the erythropoietin gene 3' hypoxia inducible enhancer element cloned into the *BamHI* site of pGEM-4Z (Promega Corp., Madison, WI) was obtained from Dr. Greg Semenza (Johns Hopkins University School of Medicine, Baltimore, MD; Semenza and Wang, 1992). The enhancer element fragment, termed herein as HREE1 (SEQ ID NO:6), was excised from the pGEM vector by cleavage with *SmaI* and *HincII* for blunt end subcloning into constructs of the present invention (below).

A construct containing 691 bp (-628 to +63) of the β-enolase (ENO3) gene was obtained from Dr. Charlotte Peterson (Veterans Administration Medical Center, University of Arkansas, Little Rock, Arkansas). A sequence containing this region is presented herein as SEQ ID NO:29.

4. Chimeric Genes and Expression Vectors of the Present Invention

The vector pGL2PV (plasmid-gene-light-promoter-vector; Promega Corp., Madison, WI), was used as the base vector for the construction of most of the plasmids described below. pGL2PV is a eukaryotic expression vector containing the SV40 early promoter upstream of the luciferase gene. The vector multiple cloning (MCS) site is just upstream of the SV40 promoter, and is designed for the insertion of DNA fragments containing enhancer sequences. pGL2BV (Promega Corp.) is similar to pGL2PV, but it does not contain an SV40 early promoter.

(i) HREE1/*luc* Constructs with Different Tissue-Specific Promoters

Plasmid pGLHRE (Figs. 1B, 2A, 3A) was made by blunt-ligating the 240 bp HREE1 fragment (SEQ ID NO:6) into the *Sma*I site of the MCS of pGL2PV (Fig. 1A).

Plasmid pGLHSA-150HRE (Fig. 2B) was made by digesting pGLHRE with *Hind*III and *Sma*I to drop out the SV40 promoter and replacing it with a 150 bp *Hind*III-*Sma*I fragment from pHSA-150CAT containing a fragment of the human skeletal actin (HSA) promoter.

Plasmid pGL$\alpha$MHC$_{86}$HRE (Fig. 2C) was made by digesting pGLHRE with *Hind*III and *Sma*I to drop out the SV40 promoter and replacing it with a 120 bp *Hind*III-*Eco*RI fragment from p$\alpha$MHC$_{86}$CAT containing 86 bp (SEQ ID NO:24) of the human $\alpha$-myosin heavy chain ($\alpha$-MHC) promoter. The *Eco*RI end of the 120 bp fragment was filled in with DNA polymerase I using standard methods (Sambrook, *et al.*) before blunt end ligation to the vector *Sma*I site.

Plasmid pGL$\alpha$MHC$_{86}$-GATA-HRE was made by cloning a 36 bp oligonucleotide (SEQ ID NO:1; described above), containing a duplicated GATA 4 box into the *Hind*III site (filled in with polymerase) of plasmid pGL$\alpha$MHC$_{86}$HRE, upstream of the 86 bp promoter fragment.

Plasmid pGLHCA$_{118}$HRE (Fig. 2D) was made by digesting pGLHRE with *Hind*III and *Sma*I to drop out the SV40 promoter and replacing it with a 188 bp *Hind*III-*Eco*RI fragment from pHCA$_{118}$CAT, containing 118 bp of the human cardiac actin (HCA) promoter plus 70 bp of actin exon 1. The *Eco*RI end of the 188 bp fragment was filled in with DNA polymerase I as above before blunt end ligation to the vector *Sma*I site.

Plasmid pGL$\alpha$MHC$_{1.2}$HRE (Fig. 3B) was made by digesting pGLHRE with *Hind*III and *Sma*I to drop out the SV40 promoter and replacing it with a 1.2 kb *Hind*III-*Eco*RI fragment from p$\alpha$MHC$_{2.0}$CAT containing 1.2 kb of the human $\alpha$-MHC promoter. The *Eco*RI end of the 1.2 kb fragment was filled in as above in prior to cloning.

(ii) PKM Promoter/*luc* Constructs

Plasmid pGLPKM$_{460}$, containing 460 bp of the rat muscle specific pyruvate kinase (PKM) gene promoter and 140 bp of the PKM coding sequence (SEQ ID NO:7), was created using polymerase chain reaction (PCR) as follows. PKM-specific primers containing endonuclease restriction sites near their 5' end were designed based on the nucleotide sequence of the PKM gene (Takenaka, *et al.*, 1989). PKM primer F (SEQ ID NO:2) contained a *Kpn*I site, while PKM primer R (SEQ ID NO:3) contained a *Xho*I site. PCR was carried out using the above primers and 1 $\mu$g of rat heart genomic DNA as a template for 25 cycles using standard procedures and a Perkin-Elmer (Norwalk, CT) DNA thermal cycler. The PCR

product (Fig. 6A) was purified by agarose gel electrophoresis, cut with *Kpn*I and *Xho*I, and cloned into *Kpn*I/*Xho*I cut pGL2BV (Fig. 6B; Promega Corp., Madison, WI), generating $pGLPKM_{460}$ (Fig. 6C).

Plasmid <u>$pGLPKM_{285}$</u> (Fig. 6E) was generated by digesting $pGLPKM_{460}$ with *Sma*I to drop out the -460 to -285 portion of the promoter, and religating the vector. <u>$pGLPKM_D$</u> (Fig. 6D) was generated by digesting $pGLPKM_{460}$ with *Sma*I to isolate the -460 to -285 portion of the promoter, and cloning that fragment into pGL2PV (Promega Corp.) that had been cut with *Sma*I.

(iii) <u>Et-1 Promoter/*luc* Constructs</u>

Plasmid <u>$pGLET\text{-}1_{700}$</u> (Fig. 7C), containing 700 bp of the human ET-1 gene promoter (SEQ ID NO:8), was created using PCR to amplify HeLa cell genomic DNA as described above. ET-1 specific primers were designed based on the promoter sequence (Inoue, *et al.*, 1989) of the ET-1 gene. The forward primer (SEQ ID NO:4) contained *Pst*I and *Kpn*I sites, while the reverse primer (SEQ ID NO:5) contained *Hind*III and *Xba*I sites. The PCR product (Fig. 7A) was purified by gel electrophoresis, cut with *Kpn*I and *Hind*III, and cloned into *Kpn*I/*Hind*III cut pGL2BV (Fig. 7B; Promega Corp.).

(iv) <u>ENO3 Promoter/*luc* Constructs</u>

Plasmid <u>$pGLENO_{628}$</u> was constructed by cloning a blunt ended genomic DNA containing an ENO3 promoter fragment (-628 to +63; SEQ ID NO:29), isolated from a lambda gt10 human genomic library, into the *Sma*I site of pGL2BV.

(v) <u>Therapeutic Gene Constructs</u>

Plasmid <u>$p\alpha MHC_{1.2}HRE\text{-}NOS$</u> (Fig. 4B) was made by digesting plasmid $pGL\alpha MHC_{1.2}HRE$ (Fig. 4A) with *Hind*III and *Eco*RV to drop out the luciferase cDNA and replacing it with a *Hind*III/*Xba*I fragment from pNOS containing a full length NOS CDNA.

Plasmid <u>$p\alpha MHC_{1.2}HRE\text{-}Bcl\text{-}2$</u> (Fig. 5B) was made by digesting pSFFV-Bcl-2 with *Sal*I, blunting the vector as described above, removing the SFFV promoter from the linearized vector with an *Eco*RI digest, and replacing the SFFV promoter with a *Sma*I/*Eco*RI fragment from $pgL\alpha MHC_{1.2}HREE$ containing the 1.2 kb αMHC promoter fragment and the 240 bp HREE1.

(vi) Other Plasmid Constructs

Plasmid p$\alpha$MHC$_{5.5}$HRE-CAT was made by inserting the 240 bp HREE1 immediately 5' of the $\alpha$MHC promoter of p$\alpha$MHC$_{5.5}$CAT.

(vi) Adenoviral Constructs

Adenoviral constructs are made using standard methods (*e.g.*, Friedman,, *et al.*, 1986; Hertz and Gerard, 1993), as follows.

Construct Ad$\alpha$MHC1.2Bcl2HREE is made by inserting a 3.34 Kb *Eco*RI/*Hind*III fragment from p$\alpha$MHC1.2-Bcl-2 (containing 1.2 Kb of the $\alpha$-MHC promoter, 1.9 Kb Bcl-2 cDNA, and 240 bp HREE1) into pAPLCMV digested with *Eco*RI and *Hind*III to drop out the CMV promoter and CAT gene. pAPLCMV, which may be obtained from Dr. Larry Kedes (University of Southern California, Los Angeles, CA; Kass-Eisler, *et al.*, 1993), is a base replication deficient adenoviral expression vector. The backbone adenoviral vector for recombination, p9M17, may also be obtained from Dr. Larry Kedes.

Recombinant pAPLCMV (pAd$\alpha$MHC1.2bcl-2HRE) and p9M17 are used to co-transfect 293 cells (ATCC) to propagate the adenovirus.

## EXAMPLE 1

### Tissue Specific Hypoxia Induced Expression *In Vitro*

Constructs pGLHRE, pGLHSA-150HRE, p$\alpha$MHC$_{5.5}$HRE-CAT, pGL$\alpha$MHC$_{1.2}$HRE, pGLHCA$_{118}$HRE and pGL-Eno$_{623}$ were tested for tissue-specific expression and hypoxia inducibility in HeLa cells, Hep G2 cells, differentiated C2C12 muscle myotubes, and cardiac myocytes.

A. Buffers and Solutions

HEPES buffered saline (HeBS; 2X solution)

16.4 g NaCl

11.9 g HEPES acid

0.21 g $Na_2HPO_4$

$H_2O$ to 1 liter

Titrate Ph to 7.05 with 5 M NaOH.

PBS Buffer

| | |
|---|---|
| 137 mM | NaCl |
| 2.7 mM | KCl |

| | |
|---|---|
| 4.3 mM | $Na_2HPO_4$ |
| 1.4 mM | $KH_2PO_4$ |

Adjust pH to 7.1.

Reconstituted Luciferase Assay Reagent (LAR)

| | |
|---|---|
| 20 mM | Tricine |
| 1.07 mM | $(MgCO_3)_4MG(OH)_2 \bullet 5H_2O$ |
| 2.67 mM | $MgSO_4$ |
| 0.1 mM | EDTA |
| 33.3 mM | DTT |
| 270 $\mu$M | coenzyme A |
| 470 $\mu$M | luciferin |
| 530 $\mu$M | ATP |

Cell Culture Lysis Reagent (CCLR; 1X Solution)

| | |
|---|---|
| 25 mM | Tris-phosphate, pH 7.8 |
| 2 mM | DTT |
| 2 mM | 1,2-diaminocyclohexane-N,N,N',N'-tetraacetic acid |
| 10% | glycerol |
| 1% | Triton X-100 |

A. Cell Transfection

HeLa cells, C2C12 myocytes, and cardiac myocytes were transfected with the indicated plasmid DNA by the standard calcium phosphate procedure (Ausubel, *et al.*).

Briefly, $10^5$ cells were plated on a 10-cm tissue culture dish and grown for 3 days. The cells were split 1:10 into 10 ml of medium one day before application of plasmid DNA. DNA for transfection was prepared by resuspending an ethanol-precipitated pellet containing 20 $\mu$g of the plasmid DNA in 450 $\mu$l $ddH_2O$ and adding 50 $\mu$l of 2.5 mM $CaCl_2$.

500 $\mu$l of 2X HeBS were added to a 15 ml conical centrifuge tube, and the solution was aerated by bubbling air with a 10 ml pipette attached to an automatic pipettor (Drummond Instruments, Fisher Scientific, Pittsburgh, PA). The DNA/$CaCl_2$ solution was added dropwise, and the resultant mixture was vortexed for 5 seconds and then allowed to sit for 20 minutes at room temperature to form precipitate.

The precipitate was added to the dishes containing the cells and the dishes were incubated overnight.

The cells were washed twice with 5 ml PBS and fed with 10 ml of complete medium. The cells were then allowed to recover for 24 hours before incubation under an atmosphere of 1.0% $O_2$, 5% $CO_2$, 94% $N_2$ for an additional 20 hours.

B. Exposure to Hypoxic Conditions

Two to three days after transfection, the cells were exposed to atmospheric oxygen (approximately 21% $O_2$, 5% $CO_2$, balance $N_2$; $pO_2$ = ~160 mmHg), or to hypoxic conditions (approximately 0.5-2.0% $O_2$, 5% $CO_2$, balance $N_2$; $pO_2$ = ~4-8 mmHg) in an environmental chamber (Anaerobic Systems, San Jose, CA, USA) which was equipped with a Nikon TMS microscope and a continuous readout oxygen electrode (Controls Katharobic, Philadelphia, PA, USA). Unless otherwise indicated, the cells were kept in the chambers for one day prior to assaying for luciferase expression.

C. Luciferase Expression

Cells transfected and treated as above were assayed for expression of the luciferase enzyme using a standard reaction protocol (Titus). Briefly, 1 ml of CCLR and 1 ml of LAR were allowed to equilibrate at room temperature. The culture medium in the dish containing the cells to be assayed was removed and the cells were rinsed twice in PBS buffer.

Approximately 300 μl of the room-temperature CCLR was added to the dish containing the cells, and the dish was incubated at room temperature for 10-15 minutes. The cells were then scraped off the bottom of the culture dish, and the solution containing the cells was transferred to a micro-centrifuge tube. The tube was centrifuged in a table-top microcentrifuge briefly (about 5 seconds) to pellet large debris.

20 μl of the supernatant (cell extract) were mixed with 100 μl of LAR at room temperature, and the light produced was measured for a period of 5 minutes, starting approximately 5 seconds after mixing, with a model #1250 LKB luminometer (BioOrbit, Gaithersburg, MD).

D. Results

Data from HeLa, C2C12, and cardiac cells are given in Table 1, below. Values, presented in arbitrary units, represent averages of three or more experiments for each condition.

Table 1

REGULATED EXPRESSION OF UBIQUITOUS- MUSCLE- AND CARDIAC-SPECIFIC PROMOTERS BY HYPOXIA

| | GL2PV | | GLHRE | | $GLHSA_{150}HRE$ | | $\alpha MHC_{1.2}HRE$ | | $GLHCA_{118}HRE$ | | $GLENO$[illegible] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | Hx | A | Hx | A | Hx | A | Hx | A | Hx | A | Hx |
| HeLa | 18 | 27 | 56 | 387 | BG | | BG | | BG | | -- | |
| C2C12 | 189 | 204 | 350 | 1680 | 46 | 278 | BG | | 48 | 248 | 320 | 1560 |
| Cardiac | 24 | 27 | 22 | 165 | 18 | 94 | 21 | 85 | 38 | 263 | 210 | 1610 |

BG - Background

Data shown in the table demonstrate that (i) none of the tested constructs carrying tissue-specific promoters are expressed above background in fibroblast-derived HeLa cells under either normal or hypoxic conditions, (ii) cells containing constructs having HREE1 and a compatible promoter (including the SV40 and tissue-specific promoters) express the reporter at levels that are $\sim 5$ to $\sim 7$ times greater under hypoxic conditions than under aerobic conditions; (iii) the HREE1 element is equally active in different cells and independent of the promoter; (iv) the $\alpha$-$MHC_{1.2}$ promoter expresses in cardiac, but not in skeletal or fibroblast - derived cells, the $HCA_{118}$ promoter expresses in both cardiac and skeletal muscle cells, but not in fibroblast-derived cells, and the $HSA_{150}$ promoter expresses in both skeletal and cardiac muscle, with stronger expression in skeletal muscle; and (v) basal (aerobic) expression from $\alpha$-$MHC_{1.2}$ $HCA_{118}$, and $HSA_{150}$ promoters is weak.

These results indicate that the HREE1 element is fully functional when fused to muscle and cardiac specific promoters and that muscle and cardiac cells are fully responsive to hypoxia in terms of the regulation of these promoters, and suggest that the $\alpha MHC_{1.2}$ propoter is an exemplary promoter for moderate levels of cardiac-specific expression.

The data also show that both the HREE present in the ENO3 promoter and HREE1, when present in constructs with the SV40 promoter, result in comparable levels of hypoxia induction in skeletal muscle cells. In cardiac cells, however, constructs containing the ENO3 HREE are expressed at significantly higher levels than those containing HREE1. Further, hypoxia increases the level of expression of the ENO3 HREE containing constructs in cardiac cells by over seven-fold, as compared with less than 5-fold in skeletal muscle cells. Plasmid $pGLENO_{628}$ confers induced expression in C2C12 myotubes and cardiac myocytes that is at least equivalent to four copies of the erythropoietin HRE (HREE1) in these cells. These results suggest that the HREE in the ENO3 promoter fragment may be a particularly effective HREE for hypoxia induction in constructs targeted with a tissue-specific promoter to cardiac or skeletal muscle cells.

## EXAMPLE 2

### Tissue Specific Hypoxia Induced Expression *In Vivo* Following Injection of Constructs into Target Animal Tissue

Constructs of the present invention were injected directly into cardiac tissue using techniques described in Buttrick, *et al.*, (1992) and Buttrick, *et al.*, (1993). Briefly, adult female Wistar rats were anesthetized with an intraperitoneal injection of chloral hydrate (0.7 ml/100 g of a 4% solution). Cardiac injections were made directly into the apex of the heart through a lateral thoractomy, after which the heart was replaced in the chest, the rats were briefly hyperventilated, and the incision closed. Fifty microliters of a DNA solution containing 2 $\mu g/\mu l$ of either pGLHRE or $pGLHCA_{118}HRE$ in 20% sucrose and 2% Evans blue were injected through a 27-gauge needle. Following injection the rats were subjected to a 20 min ischemia by cannulation of the coronary artery as described by Smith, *et al.* (1988).

Hypoxia-inducibility of vector expression was assayed as follows. Hearts were excised approximately 20 hours after the induced ischemia and the ventricles were washed with ice-cold phosphate buffered saline (PBS). The tissue was suspended in 1 ml of ice-cold PBS containing 20% sucrose and homogenized with a Polytron (Kinematica, Switzerland) for 45 sec. After centrifugation at 10,000 × g for 10 min supernatants were analyzed for luciferase expression by the assay method described above. Protein was measured using a BioRad assay kit (BioRad Laboratories, Hercules, CA).

The results of the experiments are shown in Table 2, below. Luciferase expression in hearts from rats injected with pGLHRE or $pGLHCA_{118}HRE$ and subjected to ischemia

was approximately 2-fold higher than expression in hearts from control animals injected with saline (n=3).

Table 2

ISCHEMIA INDUCIBLE EXPRESSION OF pGLHRE AND $pGLHCA_{118}HRE$ IN RAT HEART

| Plasmid | Luciferase Activity Light Units/mg Protein | |
|---|---|---|
| | Aerobic | 20 min. Ischemic |
| pGLHRE | 1180 | 2440 |
| $pGLHCA_{118}HRE$ | 88 | 127 |
| Control | 15 | 21 |

Rat hearts were injected with plasmids as described above. A 20 min. ischemia was imposed on one group (3 rats) and the other (1 control) was sham operated. Tissue samples were harvested and assayed for luciferase expression 20 hr. later.

These results indicate that the direct injection of plasmid DNA, made in accordance with the teachings of the present specification, into hearts of living mammals is effective to result in the expression of genes carried on those plasmids. Further, these results indicate that expression vectors carrying chimeric genes of the present invention are effective to result in significantly increased levels of expression in response to hypoxia caused by ischemia *in vivo*.

## EXAMPLE 3

### Stable Expression of Hypoxia Regulated NOS and Bcl-2 Genes *In Vitro*

$10^6$ C2C12 myoblasts were cotransfected with pSV2Neo (Minty and Kedes) and a test plasmid at a ratio of 1:19 (1$\mu$g pSV2Neo + 19 $\mu$g test plasmid) using standard methods (Minty and Kedes, 1986). Test plasmids were pGLHRE, $pGLHCA_{118}HRE$, $pGL\alpha MHC_{1.2}HRE$, pSFFV-Bcl-2, and pNOS-HRE. Cultures were selected on day 2 following transfection with 400 $\mu$g/ml of the neomycin drug G418 (Gibco/BRL). Colonies of cells resistant to G418 appeared after 10 to 14 days. The resistant cells were pooled. Mass cultures were assayed for the expression of luciferase as described above or by Northern blot assay (Webster, *et al.*, 1993) for the expression of Bcl-2 or NOS RNA. Stable lines were positive for expression of the transfected genes.

Mass cultures were subjected to differentiation conditions by transferring them to low mitogen medium (DMEM with 2% horse serum) and were analyzed visually for differentiation into myotubes. There was no apparent difference between transfected and control cells. Approximately 40% of cells were fused into multinucleate myotubes after 24 h in low mitogen medium. All cultures contained approximately 74% myotubes after 48h.

Reporter-transfected cells differentiated normally and respond to hypoxia with the predicted induction of reporter. NOS-transfected cells appeared normal both during the hypoxia and during subsequent reoxygenation. A stable line of C2C12 cells that constitutively over-expresses Bcl-2 (without HREE1) was also constructed as described above, and the cells showed normal growth and differentiation characteristics.

Taken together, the data presented above suggest that inclusion of HRE elements, Bcl-2 over-expression, and hypoxia-induced over-expression of NOS is not toxic to muscle cells *in vitro*. Further, the data indicate that the cells may be protected from the deleterious effects of hypoxia by the expression of therapeutic genes (*e.g.*, NOS).

## <u>EXAMPLE 4</u>

### <u>Expression of pGLPKM Plasmids under Hypoxic Conditions</u>

Plasmid $pGLPKM_{460}$ was transfected into C2C12 cells and cardiac myocytes and assayed for luciferase activity as described in Example 1. The expression of pGLPKM in both transfected C2C12 myotubes and neonatal cardiac myocytes was increased by $6 \pm 2$ fold ($n = 4$) in both cell types by incubation of the cells in an atmosphere containing 0.5 % $O_2$, 5% $CO_2$,balance $N_2$ (hypoxic conditions) relative to normal conditions, as described in Example 1.

A portion of this HRE element, obtained by digesting with *Sma*I to cut at an internal *Sma*I site, is also effective as a hypoxia response enhancer element. This fragment, termed $HREPKM_{285}$, confers hypoxia-induced expression in C2C12 myotubes and cardiac myocytes similar to that obtained with $pGLPKM_{460}$. This level of hypoxia induction is at least equivalent to that obtained using HREE1 (SEQ ID NO:6).

These results indicate that the PKM promoter fragment contained in the sequence represented as SEQ ID NO:7 contains an HRE element that is effective at enhacing the expression of chimeric genes containing the element under conditions of hypoxia.

The PKM promoter sequence has no significant homology with the erythropoietin HRE consensus, but does share a consensus sequence (SEQ ID NO:31) with the ENO3 promoter fragment (SEQ ID NO:29). This consensus, located approximately 88 bp

upstream of the transcription start site of PKM and approximately 70 bp upstream of the transcription start site of ENO3, may represent an important element for conferring enhancement of expression in response to hypoxia.

## EXAMPLE 5

### Expression of pGLET-$1_{700}$ Plasmids under Hypoxic Conditions

Plasmid pGLET-$1_{700}$ was transfected into human arterial endothelial cells as described in Example 1. The expression of pGLET-$1_{700}$ in these cells was increased 5 fold by incubation of the cells in a hypoxic atmosphere as described above. No significant induction of pGLET-$1_{700}$ was observed in any other cell types tested, including HeLa, C2C12, and cardiac myocytes. Elements contained within the 700 bp sequence have no significant homology with the erythropoietin HRE consensus.

These results indicate that the 700 bp fragment of the human ET-1 gene promoter corresponding to the sequence represented herein as SEQ ID NO:8 is effective to (i) restrict expression of genes under its control to the vascular endothelium, and (ii) confer hypoxia-inducibility on the expression of those genes. Accordingly, this fragment, in conjunction with a therapeutic or reporter gene, may be used in the methods of the present invention to both target expression to a selected tissue (vascular endothelium), and confer enhancement of expression by hypoxia.

## EXAMPLE 6

### Regulation of the Human Metallothionein IIa (hMTIIa) Promoter by Hypoxia

Three DNA fragments derived from the human MTIIa (hMTIIa) promoter, were tested in chloramphenicol acetyltransferase (CAT) reporter gene assays for hypoxia responsiveness. Fragments containing -760 bp (SEQ ID NO:32) and -345 bp (SEQ ID NO:33) of the promoter (including the first +21 bp downstream of the transcription initiation site) were cloned immediately upstream of the bacterial chloramphenicol acetyl transferase (CAT) gene in the pCAT Basic reporter vector (Promega, Madison, WI, USA), generating vectors pCAT-760 and pCAT-345, respectively. These vectors were in turn used to transfect A431 cells (ATCC Accession # CRL-7907) using the standard calcium phosphate method (Ausubel, *et al.*).

Approximately four days after transfection, the transfected cells were exposed to a selection medium comprised of Dulbecco's modified Eagle's medium (DMEM) supplement-

ed with 10% fetal bovine serum and containing 400 $\mu$g/ml G418 to select stable clones (the pCAT Basic vector contains a G417/neomycin resistance gene).

Early passages (1-10) of pooled stable clones were used in hypoxia experiments. Three hours before exposure to hypoxia, the medium bathing the cultures was changed. The dishes were placed inside specially designed aluminum chambers submerged in a 37°C water bath and attached to a 5% $CO_2/N_2$ manifold on a vacuum line (Laderoute, *et al.*, 1992). Oxygen was extracted at 37°C over 1.5 hours by 7 cycles of pumping to a fixed pressure followed by filling with 5% $CO_2/N_2$. The final $O_2$ tension in the gas phase was approximately 0.01% of atmospheric $O_2$ ($pO_2 < 0.08$ torr).

Following incubation at 37°C for the indicated time (up to 14 hours), the chambers were opened under 5% $CO_2/N_2$ in a humidified anaerobic chamber (Anaerobic Systems, San Jose, CA). Aerobic controls were incubated for an equal time period in 5% $CO_2$/air at 37°C.

Total protein for CAT assays was harvested as cell lysates using the Triton X-100 method (Laderoute, *et al.*, 1992) in the humidified anaerobic chamber following 8 or 14 hr of hypoxia. The CAT assays were conducted using standard methods (Ausubel, *et al.*). Briefly, Acyl CoA and $^{14}$C-labeled chloramphenicol were added to the cell lysates, and modified derivatives of the chloramphenicol were separated from the starting material using thin-layer chromatography. The CAT activity of the extracts was quantitated using the following formula:

$$\%\ \text{acetylated} \quad \frac{\text{counts in acetylated species}}{\text{counts in acetylated species + counts in nonacetylated chloramphenicol}}$$

Table 3, below, presents CAT activity data for the -345 bp fragment. The numbers represent the amount of CAT activity in extracts from transfected cells exposed to hypoxia divided by the CAT activity in extracts from transfected cells under normoxic conditions. The hypoxia-regulated transcriptional activation is compared with that caused by cadmium chloride (10 $\mu$M), a known activator of hMTIIa transcription (Karin and Herrlich 1989).

## Table 3

Characterization of a Hypoxia-Responsive Element (HRE) in the Promoter of the Human Metallothionein IIa Gene

| Time (hours) | Transcriptional Activation |
|---|---|
| CAT (hypoxia)/CAT air | |
| 8 | 1.8 ± 0.8[a] |
| 14 | 2.7 ± 0.2[b] |
| CAT Cd/CAT (control) | 3.9 ± 1.3[b] |

[a]Sample SD; n=4
[b]Sample SD; n=7

These results indicate that hypoxic stress can increase transcription from the hMTIIa proximal promoter. Enhancements in CAT activity relative to the aerobic controls were observed at both 8 and 14 hr of hypoxia. The levels of induction (2-3 fold) were within the same range as those found in the cadmium chloride-treated positive controls. Hypoxia responsiveness of the 760 bp construct was similar to that of the 345 bp construct.

### EXAMPLE 7

Deletion Analysis of hMTIIa Promoter

To further characterize the hMTIIa promoter, mouse C2C12 myoblasts were transiently transfected with PCR-generated nested deletion fragments of the -345 bp responsive fragment. Fragments containing -163 bp (SEQ ID NO:34) and -90 bp (SEQ ID NO:35) of the hMTIIa promoter (including the first +21 bp downstream of the transcription initiation site) were inserted immediately upstream of the luciferase reporter gene of the pGL2 plasmid (Promega, Madison, WI), generating pGL2-163 and pGL2-90, respectively. The plasmids were used to transiently transfect the C2C12 cells as described above.

The transfected cells were subjected to hypoxia treatment and cell extracts were made as described above. Luciferase activity of cell extracts was measured using a standard assay (Ausubel, *et al.*). Briefly, ATP and the substrate luciferin were added to the lysate in a luminometer, and total light output was measured. The amount of light was proportional to the amount of luciferase present in the extracts.

Extracts from both pGL2-163- and pGL2-90-transfected cells showed significant upregulation of luciferase activity under hypoxic conditions, with levels of induction

(approximately 3.0-fold) similar to those observed in Example 6, above. These results suggest that at least one HRE is contained in the proximal 90 bp fragment (SEQ ID NO:35) of the hMTIIa promoter.

## EXAMPLE 8

### Induction of Toxic Genes by hMTIIa HRE *In Vitro*

The luciferase coding sequence in the pGL3-Basic promoter vector (Promega) is excised as a *NcoI/XbaI* fragment and replaced with a double-stranded PCR-generated DNA fragment encoding human tumor necrosis factor (hTNF) (SEQ ID NO:37; Shirai, *et al.*). TNF is a growth factor that rapidly and induces programmed cell death or apoptosis (Cleveland and Ihle, 1995), and is not known to be induced by hypoxic stress. The -90 bp hMTIIa fragment (SEQ ID NO:35) is inserted immediately upstream of the TNF gene, resulting in construct hMTIIa-HRE-TNF.

The construct is used to transfect both C2C12 cells (transient transfection) and A431 cells (stable transfection) as described above. Transfected cells are then subjected to either normoxic or hypoxic conditions for periods of time ranging from 8 to 24 hr as described above, and induced cytotoxicity of the TNF protein is evaluated using a standard clonogenic assay (*e.g.*, as described in Kowk and Sutherland, 1989). Briefly, several dilutions, 3 replicates per dilution, are plated for each time point, and the cells are incubated undisturbed in a humidified 37°C incubator for 10-20 days. Cell colonies are stained with methylene blue and colonies with 30 or more cells are scored. Northern and Western analyses are performed immediately after hypoxic treatment to determine induction of TNF.

## EXAMPLE 9

### Hypoxia-Mediated TNF induction and Tumor Control in an Animal Xenograft Model

To determine the stage at which tumors develop a substantial hypoxic portion, nude mice (Taconic, Germantown, NY, USA) ranging in age from 4-5 weeks, are injected by subcutaneous (s.c.) unilateral injections of about $5 \times 10^6$ exponentially growing untransfected A431 cells into the dorsum of the right side. Hypoxic regions are identified using a derivative of 2-nitroimidazole etanidazole, the fluorinated bioreductive compound 2-(2-nitro-1H-imidazol-1-yl)-N-(2,2,3,3,3-pentafluoropropyl)acetamide (EF5; obtained from Dr. Cameron Koch, Department of Radiation and Oncology, School of Medicine, University of Pennsylvania; Lord, *et al.*, 1993). Etanidazole forms covalent bonds to cellular macromolecules after bioreduction at low oxygen tensions (Lord, *et al.*, 1993). Monoclonal

antibodies raised against these nonphysiological adducts (Lord, *et al.*, 1993) are employed using standard immunohistochemistry to image hypoxic regions in serial frozen sections (7$\mu$m) from tumors harvested twice per week.

A. Testing Reporter Constructs *In Vivo*

Reporter gene constructs containing the luciferase gene under the control of an HRE from the hMTIIa promoter are made as described above and used to stably transfect A431 cells.

Experiments are conducted using three groups of mice, each group injected as described above with one of three types of cells: 1) untransfected cells, 2) stable transfectants containing the empty pGL2 vector and 3) stable transfectants containing the hMTIIA-HRE-pGL2 construct. Groups 1 and 2 are used as negative controls.

The tumors are allowed to grow to a stage at which they contain a substantial hypoxic portion, determined as described above. The mice are then sacrificed, tumors are removed and cut on a cryostat, and the resulting frozen sections are analyzed for luciferase activity and EF5 staining. The degree of overlap between the luciferase activity and EF5 staining in group 3 mice relates to the potential effectiveness of such an HRE-containing construct in a tumor *in vivo*.

B. Testing Toxic Constructs *In Vivo*

These experiments are conducted as described above, except that they employ A431 cells transfected with the hMTIIa-HRE-TNF construct or the empty vector (missing both the hMTIIa-HRE and the TNF cDNA). Frozen sections are scored for apoptosis using the "APOTAG" kit (Oncor, Gaitherburg, MD.). Effectiveness of the construct is measured by increased apoptosis in the hypoxic regions of tumors containing the transfected hMTIIa-HRE-TNF construct as compared with tumors containing the empty vector.

While the invention has been described with reference to specific methods and embodiments, it is appreciated that various modifications and changes may be made without departing from the invention.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SRI International

(ii) TITLE OF INVENTION: Tissue Specific Hypoxia Regulated Therapeutic Constructs

(iii) NUMBER OF SEQUENCES: 37

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Dehlinger & Associates
(B) STREET: 350 Cambridge Avenue, Suite 250
(C) CITY: Palo Alto
(D) STATE: CA
(E) COUNTRY: USA
(F) ZIP: 94306

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/IB95/00996
(B) FILING DATE: 13-NOV-1995
(C) CLASSIFICATION:

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(A) APPLICATION NUMBER: US 08/365,486
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(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Sholtz, Charles K.
(B) REGISTRATION NUMBER: 38,615
(C) REFERENCE/DOCKET NUMBER: 8255-0018.41

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (415) 324-0880
(B) TELEFAX: (415) 324-0960

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: GATA4 Enhancer

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAAAGGGCCG ATGGGCAGAT AGAGGAGAGA CAGGA 35

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: PKM primer F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

AATTGGTACC CGGGCGAGCG CCGGGAGGGT GGA 33

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: PKM primer R

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAACTCGAG GCACTATGGC ATTGGCTCTG GG 32

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ET-1 primer F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TATATCTGCA GGTACCGATA GGGAAAAGAC TGGCATGTGC C 41

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: ET-1 primer R

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TATATAAGCT TCTAGAGACC CGTTCGCCTG GCGCGCAGAT GCA 43

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 240 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: HREE1 (Hypoxia responsive enhancer element 1)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCCGGGATC 60

CCGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCCGGGATC 120

CCGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCCGGGATC 180

CCGGCCCTAC GTGCTGTCTC ACACAGCCTG TCTGACCTCT CGACCTACCG GCCGATCCCG 240

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: sequence containing PKM promoter frag.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAGTCACCGG GCGGGGCTGG AGGAATGTCC GGGACCTATA AATCTGGGCA ACGCCCTGGT 60

AGGCCAGGGC AGATGGGGCA CCTGGGCAGA ATTCCAAAAT GGGATTATGT AGCCTCTGAG 120

GTCCTAAAGC AACAGGTGGC GGACCACCCG GGGATCTAGG GGTGGTGGCG GCGGTGGACC 180

CGAGGGCGGG TCCTGCCTCC TCACCACTTC CCCATTGGCC ATCAGAATGA CCCATGCGCA 240

ATTTTGGTTT GCAATGTCCT TCCGCCACGG AAGGTAGTCC CCCTCAAAAG GGCAACCTGC 300

TTGTCCCGCC TACCCTGCGA CTCTCTCAGA AGGTGCGGGT GCCTGTTGAG AGGCGGGGCT 360

CTGCTAGCTC CTGCCCGGAT TGGGCGAGGG GCGGGGCTGC GGAGGGATTG CGGCGGCCCG 420

CAGCAGTGAT AACCTTGAGG CCCAGTCTGC GCAGCCCCGC ACAGCAGCGA CCCGTCCTAA 480

GTCGACAGAC GTCCTCTTTA GGTATTGCAA CAGGATCTGA AGTACGCCCG AGGTGAGCGG 540

GGAGAACCTT TGCCATTCTC 560

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 713 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Sequence containing ET-1 promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GATAGGGAAA AGACTGGCAT GTGCCTAAAC GAGCTCTGAT GTTATTTTTA AGCTCCCTTT 60

CTTGCCAATC CCTCACGGAT CTTTCTCCGA TAGATGCAAA GAACTTCAGC AAAAAAGACC 120

CGCAGGAAGG GGCTTGAAGA GAAAAGTACG TTGATCTGCC AAAATAGTCT GACCCCCAGT 180

AGTGGGCAGT GACGAGGGAG AGCATTCCCT TGTTTGACTG AGACTAGAAT CGGAGAGACA 240

TAAAAGGAAA ATGAAGCGAG CAACAATTAA AAAAAATTCC CCGCACACAA CAATACAATC 300

TATTTAAACT GTGGCTCATA CTTTTCATAC CAATGGTATG ACTTTTTTTC TGGAGTCCCC 360

TCTTCTGATT CTTGAACTCC GGGGCTGGCA GCTTGCAAAG GGGAAGCGGA CTCCAGCACT 420

GCACGGGCAG GTTTAGCAAA GGTCTCTAAT GGGTATTTTC TTTTTCTTAG CCCTGCCCCC 480

GAATTGTCAG ACGGCGGGCG TCTGCTTCTG AAGTTAGCAG TGATTTCCTT TCGGGCCTGG 540

CTTATCTCCG GCTGCACGTT GCCTGTTGGT GACTAATAAC ACAATAACAT TGTCTGGGGC 600

TGGAATAAAG TCGGAGCTGT TTACCCCCAC TCTAATAGGG GTTCAATATA AAAAGCCGGC 660

AGAGAGCTGT CCAAGTCAGA CGCGCCTCTG CATCTGCGCC AGGCGAACGG GTC 713

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 118 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: HCA118 promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

```
CGAAGGGGAC CAAATAAGGC AAGGTGGCAG ACCGGGCCCC CCACCCCTGC CCCCGGCTGC      60

TCCAACTGAC CCTGTCCATC AGCGTTCTAT AAAGCGGCCC TCCTGGAGCC AGCCACCC       118
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(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1588 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Rat alpha MHC promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
GAATTCTCTT ACTATCAAAG GGAAACTGAG TCATGCACCT GCAAAATGAA TGCCCTCCCT      60

GGACATCATG ACTTTGTCCC TGGGGAGCCA GCACTGTGGA ACTCCAGGTC TGAGAGTAGG     120

AGGCACCCCT CAGCCTGAAG CTGTGCAGAT AGCTAGGGTG TAAAAGAGGG AAGGGGGGAG     180

GCTGGAATGG GAGCTTGTGT GTTCGGAGAC AGGGGACAAA TATTAGGCCC GTAAGAGAAG     240

GTGACCCTTA CCCAGTGTGT TCAACTCAGC CTTTCAGATT AAAAATAACT AAGGTAAGGG     300

CCATGTGGGT AGGGGAGGTG GTGTGAGACG GTCCTGTCTC TCCTCTATCT GCCCATCGGC     360

CCTTTGGGGA GGAGGAAATG TGCCCAAGGA CTAAAAAAGG CCTGGAGCCA GAGGGGCTAG     420

GGCTAAGCAG ACCTTTCATG GGCAAACCTC AGGGCTGCTG TCCTCCTGTC ACCTCCAGAG     480

CCAAGGGATC AAAGGAGGAG GAGCCAGACA GGAGGGATGG GAGGGAGGGT CCCAGCAGAT     540

GACTCCAAAT TTAGGCAGCA GGCACGCGGA ATGAGCTATA AAGGGGCTGG AGCGCTGAGA     600

GCTGTCAGAC CGAGATTTCT CCATCCCAAG TAAGAAGGAG TTTAGCGTGG GGGCTCTCCA     660

ACCGCACCAG ACCTGTCCCA CCTAGAGGGA AAGTGTCTTC CCTGGAAGTG GGCTCCTCCC     720

ACCGGCCTGG GAAGATTCCT CGGTGGGCAG GATGTTCTAC TGGATGCCCC TTCCCTTCCA     780
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CTGCCTCCTC CCTCCCTTGT CTTGATTAAT CTTGGCTCTT AGTGTTCAGA AAGATTTGCC 840

CGGTGCTGTC TACTCCATCT GTCTCTACTC TCTCTGCCTT GCCTTCTTGT GTGTTCTCCT 900

TTTCCACGTG TTTCTCACTC CACTGCCTCC CCCCCCCCCT TCATTTTTAT CCTTCCTTTC 960

TTTCTGTGTC AGAATGCTGG GAATCAAACC CAGGGCTTCA TACACGTCAA GTAAGCAATC 1020

TCCCAGTGAG TCAAAGCTTT AATCCTCTGG GTGCTGTCTT ACCGAGCCTC ACTCCCTGTC 1080

TTGTCCTGTT CCGTCCTAGT CAGGATCTCT GGTCCGTCTC TCAGCTTCTG CTACTCCTCT 1140

CCCTGCCTGC TCTTCTCTCC GTCCAGCTGC ACCTCTGTGG CGCTCATTCC AGCCGTGGTC 1200

CAAATTCTCT GTGAAAAGAT TAACCGGGTG AGAATGCCCC CAGTTTCCCC TGTAGACAGC 1260

AGATCATGAT TTTCCCCAGA AGCCAGACTT CCAGCGCCCG CCCTCTGCCC AGCAACTTGA 1320

CACTCTTAGC AAACTTCAGC CACCCTTCCC CCACATAGAC CAAGTCTTGC AGAGAGCCTT 1380

CCTTCAGATG ACTTCGAGTT CTTGCAAAGG AAGGAGAACT CTTTGTGGCG GGGAAGCAGG 1440

CACTTTACAC GGAGTCTGAC GGGAGGTCAT AGGCTATGGC ATAGCAGAGG CAGGGAGGTG 1500

GTGGAATTGG ACTTCGCGCA GAAGCTAAGC ACACACCAGG AATGACATAT CCCTCCTATC 1560

TCCCCCATAA GAGTTTAAGA GTGACAGG 1588

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1679 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Mouse alpha MHC promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAATTCTCTT ACTATCAAAG GGAAACTGAG TCGTGCACCT GCAAAGTGGA TGCTCTCCCT 60

AGACATCATG ACTTTGTCTC TGGGGAGCCA GCACTGTGGA ACTTCAGGTC TGAGAGAGTA 120

GGAGGCTCCC CTCAGCCTGA AGCTATGCAG ATAGCCAGGG TTGAAAGGGG GAAGGGAGAG 180

CCTGGGATGG GAGCTTGTGT GTTGGAGGCA GGGGACAGAT ATTAAGCCTG GAAGAGAAGG 240

TGACCCTTAC CCAGTTGTTC AACTCACCCT TCAGATTAAA AATAACTGAG GTAAGGGCCT 300

GGGTAGGGGA GGTGGTGTGA GACGCTCCTG TCTCTCCTCT ATCTGCCCAT CGGCCCTTTG 360

GGGAGGAGGA ATGTGCCCAA GGACTAAAAA AAGGCCATGG AGCCAGAGGG GCGAGGGCAA 420

CAGACCTTTC ATGGGCAAAC CTTGGGGCCC TGCTGTCCTC CTGTCACCTC CAGAGCCAAG 480

GGATCAAAGG AGGAGGAGCC AGGACAGGAG GGAAGTGGGA GGGAGGGTCC CAGCAGAGGA 540

CTCCAAATTT AGGCAGCAGG CATATGGGAT GGGATATAAA GGGGCTGGAG CACTGAGAGC 600

TGTCAGAGAT TTCTCCAACC CAGGTAAGAG GGAGTTTCGG GTGGGGGCTC TTCACCCACA 660
CCAGACCTCT CCCCACCTAG AAGGAAACTG CCTTTCCTGG AAGTGGGGTT CAGGCCGGTC 720
AGAGATCTGA CAGGGTGGCC TTCCACCAGC CTGGGAAGTT CTCAGTGGCA GGAGGTTTCC 780
ACAAGAAACA CTGGATGCCC CTTCCCTTAC GCTGTCTTCT CCATCTTCCT CCTGGGGATG 840
CTCCTCCCCG TCTTGGTTTA TCTTGGCTCT TCGTCTTCAG CAAGATTTGC CCTGTGCTGT 900
CCACTCCATC TTTCTCTACT GTCTCCGTGC CTTGCCTTGC CTTCTTGCGT GTCCTTCCTT 960
TCCACCCATT TCTCACTTCA CCTTTTCTCC CCTTCTCATT TGTATTCATC CTTCCTTCCT 1020
TCCTTCCTTC CTTCCTTCCT TCCTTCCTTC CTTCCTTTCT CCCTTCCTTC CTTCCTTCCT 1080
TCCTTCCTTC CTTCCTTCCT TCCTGTGTCA GAGTGCTGAG AATCACACCT GGGGTTCCCA 1140
CCCTTATGTA AACAATCTTC CAGTGAGCCA CAGCTTCAGT GCTGCTGGGT GCTCTCTTAC 1200
CTTCCTCACC CCCTGGCTTG TCCTGTTCCA TCCTGGTCAG GATCTCTAGA TTGGTCTCCC 1260
AGCCTCTGCT ACTCCTCTTC CTGCCTGTTC CTCTCTCTGT CCAGCTGCGC CACTGTGGTG 1320
CCTCGTTCCA GCTGTGGTCC ACATTCTTCA GGATTCTCTG AAAAGTTAAC CAGGTGAGAA 1380
TGTTTCCCCT GTAGACAGCA GATCACGATT CTCCCGGAAG TCAGGCTTCC AGCCCTCTCT 1440
TTCTCTGCCC AGCTGCCCGG CACTCTTAGC AAACCTCAGG CACCCTTACC CCACATAGAC 1500
CTCTGACAGA GAAGCAGGCA CTTTACATGG AGTCCTGGTG GGAGAGCCAT AGGCTACGGT 1560
GTAAAAGAGG CAGGGAAGTG GTGGTGTAGG AAAGTCAGGA CTTCACATAG AAGCCTAGCC 1620
CACACCAGAA ATGACAGACA GATCCCTCCT ATCTCCCCCA TAAGAGTTTG AGTGACAGA 1679

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5057 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: rat bNOS cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 349..4638

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ACGTCTGACA AGCTGGTGAC CAAGATGCCC AGAGACTAGA CCCTATGCTT GTGAGTCACA 60
GTCATCAGAC ACGGCAAACC TCCAGTCTTC CTGACCTGTT GCTTAGGGAC ACATCCCGTT 120
GCTGCCCCTG ACGTCTGCCT GGTCAACCTT GACTTCCTTT GAGAGTAAGG AAGGGGGCGG 180
GGACACGTTG AAATCATGCC ACCCAAGGCC GAATCGGAAT GAGCAGATGA CGCCAAGTTG 240

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ACGTCAAAGA CAGAGGCGAC AGAAACTCTG CAGCCAGCTC TTGCCCCCGA GGAGCTCAGG   300

TTCCTGCAGG AGTCATTTTA GCTTAGTCTT CTGAAGGACA CAGATACC ATG GAA GAG     357
                                                     Met Glu Glu
                                                      1

AAC ACG TTT GGG GTT CAG CAG ATC CAA CCC AAT GTA ATT TCT GTT CGT      405
Asn Thr Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile Ser Val Arg
     5                  10                  15

CTC TTC AAA CGC AAA GTG GGA GGT CTG GGC TTC CTG GTG AAG GAA CGG      453
Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val Lys Glu Arg
 20                  25                  30                  35

GTC AGC AAG CCT CCC GTG ATC ATC TCA GAC CTG ATT CGA GGA GGT GCT      501
Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg Gly Gly Ala
                 40                  45                  50

GCG GAG CAG AGC GGC CTT ATC CAA GCT GGA GAC ATC ATT CTC GCA GTC      549
Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile Leu Ala Val
             55                  60                  65

AAC GAT CGG CCC TTG GTA GAC CTC AGC TAT GAC AGT GCC CTG GAG GTT      597
Asn Asp Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala Leu Glu Val
         70                  75                  80

CTC AGG GGC ATT GCC TCT GAG ACC CAC GTG GTC CTC ATT CTG AGG GGC      645
Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile Leu Arg Gly
     85                  90                  95

CCT GAG GGC TTC ACT ACA CAT CTG GAG ACC ACC TTC ACA GGG GAT GGA      693
Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr Gly Asp Gly
100                 105                 110                 115

ACC CCC AAG ACC ATC CGG GTG ACC CAG CCC CTC GGT CCT CCC ACC AAA      741
Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro Pro Thr Lys
                120                 125                 130

GCC GTC GAT CTG TCT CAC CAG CCT TCA GCC AGC AAA GAC CAG TCA TTA      789
Ala Val Asp Leu Ser His Gln Pro Ser Ala Ser Lys Asp Gln Ser Leu
            135                 140                 145

GCA GTA GAC AGA GTC ACA GGT CTG GGT AAT GGC CCT CAG CAT GCC CAA      837
Ala Val Asp Arg Val Thr Gly Leu Gly Asn Gly Pro Gln His Ala Gln
        150                 155                 160

GGC CAT GGG CAG GGA GCT GGC TCA GTC TCC CAA GCT AAT GGT GTG GCC      885
Gly His Gly Gln Gly Ala Gly Ser Val Ser Gln Ala Asn Gly Val Ala
    165                 170                 175

ATT GAC CCC ACG ATG AAA AGC ACC AAG GCC AAC CTC CAG GAC ATC GGG      933
Ile Asp Pro Thr Met Lys Ser Thr Lys Ala Asn Leu Gln Asp Ile Gly
180                 185                 190                 195

GAA CAT GAT GAA CTG CTC AAA GAG ATA GAA CCT GTG CTG AGC ATC CTC      981
Glu His Asp Glu Leu Leu Lys Glu Ile Glu Pro Val Leu Ser Ile Leu
                200                 205                 210

AAC AGT GGG AGC AAA GCC ACC AAC AGA GGG GGA CCA GCC AAA GCA GAG     1029
Asn Ser Gly Ser Lys Ala Thr Asn Arg Gly Gly Pro Ala Lys Ala Glu
            215                 220                 225

ATG AAA GAC ACA GGA ATC CAG GTG GAC AGA GAC CTC GAT GGC AAA TCG     1077
Met Lys Asp Thr Gly Ile Gln Val Asp Arg Asp Leu Asp Gly Lys Ser
        230                 235                 240

CAC AAA GCT CCG CCC CTG GGC GGG GAC AAT GAC CGC GTC TTC AAT GAC     1125
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His Lys Ala Pro Pro Leu Gly Gly Asp Asn Asp Arg Val Phe Asn Asp
    245                 250                 255

CTG TGG GGG AAG GAC AAC GTT CCT GTG ATC CTT AAC AAC CCG TAT TCA      1173
Leu Trp Gly Lys Asp Asn Val Pro Val Ile Leu Asn Asn Pro Tyr Ser
260                 265                 270                 275

GAG AAG GAA CAG TCC CCT ACC TCG GGG AAA CAG TCT CCC ACC AAG AAC      1221
Glu Lys Glu Gln Ser Pro Thr Ser Gly Lys Gln Ser Pro Thr Lys Asn
                280                 285                 290

GGC AGC CCT TCC AGG TGC CCC CGT TTC CTC AAG GTC AAG AAC TGG GAG      1269
Gly Ser Pro Ser Arg Cys Pro Arg Phe Leu Lys Val Lys Asn Trp Glu
            295                 300                 305

ACG GAC GTG GTC CTC ACC GAC ACC CTG CAC CTG AAG AGC ACA CTG GAA      1317
Thr Asp Val Val Leu Thr Asp Thr Leu His Leu Lys Ser Thr Leu Glu
        310                 315                 320

ACG GGG TGC ACA GAG CAC ATT TGC ATG GGC TCG ATC ATG CTG CCT TCC      1365
Thr Gly Cys Thr Glu His Ile Cys Met Gly Ser Ile Met Leu Pro Ser
    325                 330                 335

CAG CAC ACG CGG AAG CCA GAA GAT GTC CGC ACA AAG GAC CAG CTC TTC      1413
Gln His Thr Arg Lys Pro Glu Asp Val Arg Thr Lys Asp Gln Leu Phe
340                 345                 350                 355

CCT CTA GCC AAA GAA TTT CTC GAC CAA TAC TAC TCA TCC ATT AAG AGA      1461
Pro Leu Ala Lys Glu Phe Leu Asp Gln Tyr Tyr Ser Ser Ile Lys Arg
                360                 365                 370

TTT GGC TCC AAG GCC CAC ATG GAC AGG CTG GAG GAG GTG AAC AAG GAG      1509
Phe Gly Ser Lys Ala His Met Asp Arg Leu Glu Glu Val Asn Lys Glu
            375                 380                 385

ATT GAA AGC ACC AGC ACC TAC CAG CTC AAG GAC ACC GAG CTC ATC TAT      1557
Ile Glu Ser Thr Ser Thr Tyr Gln Leu Lys Asp Thr Glu Leu Ile Tyr
        390                 395                 400

GGC GCC AAG CAT GCC TGG CGG AAC GCC TCT CGA TGT GTG GGC AGG ATC      1605
Gly Ala Lys His Ala Trp Arg Asn Ala Ser Arg Cys Val Gly Arg Ile
    405                 410                 415

CAG TGG TCC AAG CTG CAG GTG TTC GAT GCC CGA GAC TGC ACC ACA GCC      1653
Gln Trp Ser Lys Leu Gln Val Phe Asp Ala Arg Asp Cys Thr Thr Ala
420                 425                 430                 435

CAC GGC ATG TTC AAC TAC ATC TGT AAC CAT GTC AAG TAT GCC ACC AAC      1701
His Gly Met Phe Asn Tyr Ile Cys Asn His Val Lys Tyr Ala Thr Asn
                440                 445                 450

AAA GGG AAT CTC AGG TCG GCC ATC ACG ATA TTC CCT CAG AGG ACT GAC      1749
Lys Gly Asn Leu Arg Ser Ala Ile Thr Ile Phe Pro Gln Arg Thr Asp
            455                 460                 465

GGC AAA CAT GAC TTC CGA GTG TGG AAC TCG CAG CTC ATC CGC TAC GCG      1797
Gly Lys His Asp Phe Arg Val Trp Asn Ser Gln Leu Ile Arg Tyr Ala
        470                 475                 480

GGC TAC AAG CAG CCA GAT GGC TCT ACC TTG GGG GAT CCA GCC AAT GTG      1845
Gly Tyr Lys Gln Pro Asp Gly Ser Thr Leu Gly Asp Pro Ala Asn Val
    485                 490                 495

CAG TTC ACG GAG ATC TGT ATA CAG CAG GGC TGG AAA GCC CCA AGA GGC      1893
Gln Phe Thr Glu Ile Cys Ile Gln Gln Gly Trp Lys Ala Pro Arg Gly
500                 505                 510                 515
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CGC TTC GAC GTG CTG CCT CTC CTG CTT CAG GCC AAT GGC AAT GAC CCT 1941
Arg Phe Asp Val Leu Pro Leu Leu Leu Gln Ala Asn Gly Asn Asp Pro
520 525 530

GAG CTC TTC CAG ATC CCC CCA GAG CTG GTG CTG GAA GTG CCC ATC AGG 1989
Glu Leu Phe Gln Ile Pro Pro Glu Leu Val Leu Glu Val Pro Ile Arg
535 540 545

CAC CCC AAG TTC GAC TGG TTT AAG GAC CTG GGG CTC AAA TGG TAT GGC 2037
His Pro Lys Phe Asp Trp Phe Lys Asp Leu Gly Leu Lys Trp Tyr Gly
550 555 560

CTC CCC GCT GTG TCC AAC ATG CTG CTG GAG ATC GGG GGC CTG GAG TTC 2085
Leu Pro Ala Val Ser Asn Met Leu Leu Glu Ile Gly Gly Leu Glu Phe
565 570 575

AGC GCC TGT CCC TTC AGC GGC TGG TAC ATG GGC ACA GAG ATC GGC GTC 2133
Ser Ala Cys Pro Phe Ser Gly Trp Tyr Met Gly Thr Glu Ile Gly Val
580 585 590 595

CGT GAC TAC TGT GAC AAC TCT CGA TAC AAC ATC CTG GAG GAA GTA GCC 2181
Arg Asp Tyr Cys Asp Asn Ser Arg Tyr Asn Ile Leu Glu Glu Val Ala
600 605 610

AAG AAG ATG GAT TTG GAC ATG AGG AAG ACC TCG TCC CTC TGG AAG GAC 2229
Lys Lys Met Asp Leu Asp Met Arg Lys Thr Ser Ser Leu Trp Lys Asp
615 620 625

CAA GCA CTG GTG GAG ATC AAC ATT GCT GTT CTA TAT AGC TTC CAG AGT 2277
Gln Ala Leu Val Glu Ile Asn Ile Ala Val Leu Tyr Ser Phe Gln Ser
630 635 640

GAC AAG GTG ACC ATC GTT GAC CAC CAC TCT GCC ACG GAG TCC TTC ATC 2325
Asp Lys Val Thr Ile Val Asp His His Ser Ala Thr Glu Ser Phe Ile
645 650 655

AAA CAC ATG GAG AAT GAA TAC CGC TGC AGA GGG GGC TGC CCC GCC GAC 2373
Lys His Met Glu Asn Glu Tyr Arg Cys Arg Gly Gly Cys Pro Ala Asp
660 665 670 675

TGG GTG TGG ATT GTG CCT CCC ATG TCG GGC AGC ATC ACC CCT GTC TTC 2421
Trp Val Trp Ile Val Pro Pro Met Ser Gly Ser Ile Thr Pro Val Phe
680 685 690

CAC CAG GAG ATG CTC AAC TAT AGA CTC ACC CCG TCC TTT GAA TAC CAG 2469
His Gln Glu Met Leu Asn Tyr Arg Leu Thr Pro Ser Phe Glu Tyr Gln
695 700 705

CCT GAT CCA TGG AAC ACC CAC GTG TGG AAG GGC ACC AAC GGG ACC CCC 2517
Pro Asp Pro Trp Asn Thr His Val Trp Lys Gly Thr Asn Gly Thr Pro
710 715 720

ACG AAG CGG CGA GCT ATC GGC TTT AAG AAA TTG GCA GAG GCC GTC AAG 2565
Thr Lys Arg Arg Ala Ile Gly Phe Lys Lys Leu Ala Glu Ala Val Lys
725 730 735

TTC TCA GCC AAG CTA ATG GGG CAG GCC ATG GCC AAG AGG GTC AAG GCG 2613
Phe Ser Ala Lys Leu Met Gly Gln Ala Met Ala Lys Arg Val Lys Ala
740 745 750 755

ACC ATT CTC TAC GCC ACA GAG ACA GGC AAA TCA CAA GCC TAT GCC AAG 2661
Thr Ile Leu Tyr Ala Thr Glu Thr Gly Lys Ser Gln Ala Tyr Ala Lys
760 765 770

ACC CTG TGT GAG ATC TTC AAG CAC GCC TTC GAT GCC AAG GCA ATG TCC 2709
Thr Leu Cys Glu Ile Phe Lys His Ala Phe Asp Ala Lys Ala Met Ser
775 780 785

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ATG GAG GAG TAT GAC ATC GTG CAC CTG GAG CAC GAA GCC CTG GTC TTG      2757
Met Glu Glu Tyr Asp Ile Val His Leu Glu His Glu Ala Leu Val Leu
        790                 795                 800

GTG GTC ACC AGC ACC TTT GGC AAT GGA GAC CCC CCT GAG AAC GGG GAG      2805
Val Val Thr Ser Thr Phe Gly Asn Gly Asp Pro Pro Glu Asn Gly Glu
    805                 810                 815

AAA TTC GGC TGT GCT TTA ATG GAG ATG AGG CAC CCC AAC TCT GTG CAG      2853
Lys Phe Gly Cys Ala Leu Met Glu Met Arg His Pro Asn Ser Val Gln
820                 825                 830                 835

GAG GAG AGA AAG AGC TAC AAG GTC CGA TTC AAC AGC GTC TCC TCC TAT      2901
Glu Glu Arg Lys Ser Tyr Lys Val Arg Phe Asn Ser Val Ser Ser Tyr
                840                 845                 850

TCT GAC TCC CGA AAG TCA TCG GGC GAC GGA CCC GAC CTC AGA GAC AAC      2949
Ser Asp Ser Arg Lys Ser Ser Gly Asp Gly Pro Asp Leu Arg Asp Asn
            855                 860                 865

TTT GAA AGT ACT GGA CCC CTG GCC AAT GTG AGG TTC TCA GTG TTC GGC      2997
Phe Glu Ser Thr Gly Pro Leu Ala Asn Val Arg Phe Ser Val Phe Gly
        870                 875                 880

CTC GGC TCT CGG GCG TAC CCC CAC TTC TGT GCC TTT GGG CAT GCG GTG      3045
Leu Gly Ser Arg Ala Tyr Pro His Phe Cys Ala Phe Gly His Ala Val
    885                 890                 895

GAC ACC CTC CTG GAG GAA CTG GGA GGG GAG AGG ATT CTG AAG ATG AGG      3093
Asp Thr Leu Leu Glu Glu Leu Gly Gly Glu Arg Ile Leu Lys Met Arg
900                 905                 910                 915

GAG GGG GAT GAG CTT TGC GGA CAG GAA GAA GCT TTC AGG ACC TGG GCC      3141
Glu Gly Asp Glu Leu Cys Gly Gln Glu Glu Ala Phe Arg Thr Trp Ala
                920                 925                 930

AAG AAA GTC TTC AAG GCA GCC TGT GAT GTG TTC TGC GTG GGG GAT GAC      3189
Lys Lys Val Phe Lys Ala Ala Cys Asp Val Phe Cys Val Gly Asp Asp
            935                 940                 945

GTC AAC ATC GAG AAG CCG AAC AAC TCC CTC ATT AGC AAT GAC CGA AGC      3237
Val Asn Ile Glu Lys Pro Asn Asn Ser Leu Ile Ser Asn Asp Arg Ser
        950                 955                 960

TGG AAG AGG AAC AAG TTC CGC CTC ACG TAT GTG GCG GAA GCT CCA GAT      3285
Trp Lys Arg Asn Lys Phe Arg Leu Thr Tyr Val Ala Glu Ala Pro Asp
    965                 970                 975

CTG ACC CAA GGT CTT TCC AAT GTT CAC AAA AAA CGA GTC TCG GCT GCT      3333
Leu Thr Gln Gly Leu Ser Asn Val His Lys Lys Arg Val Ser Ala Ala
980                 985                 990                 995

CGA CTC CTC AGC CGC CAA AAC CTG CAA AGC CCT AAG TTC AGC CGA TCG      3381
Arg Leu Leu Ser Arg Gln Asn Leu Gln Ser Pro Lys Phe Ser Arg Ser
                1000                1005                1010

ACC ATC TTC GTG CGT CTC CAC ACC AAC GGG AAT CAG GAG CTG CAG TAC      3429
Thr Ile Phe Val Arg Leu His Thr Asn Gly Asn Gln Glu Leu Gln Tyr
            1015                1020                1025

CAG CCA GGG GAC CAC CTG GGT GTC TTC CCC GGC AAC CAC GAG GAC CTC      3477
Gln Pro Gly Asp His Leu Gly Val Phe Pro Gly Asn His Glu Asp Leu
        1030                1035                1040

GTG AAT GCA CTC ATT GAA CGG CTG GAG GAT GCA CCG CCT GCC AAC CAC      3525
Val Asn Ala Leu Ile Glu Arg Leu Glu Asp Ala Pro Pro Ala Asn His
    1045                1050                1055
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GTG GTG AAG GTG GAG ATG CTG GAG GAG AGG AAC ACT GCT CTG GGT GTC 3573
Val Val Lys Val Glu Met Leu Glu Glu Arg Asn Thr Ala Leu Gly Val
1060 1065 1070 1075

ATC AGT AAT TGG AAG GAT GAA TCT CGC CTC CCA CCC TGC ACC ATC TTC 3621
Ile Ser Asn Trp Lys Asp Glu Ser Arg Leu Pro Pro Cys Thr Ile Phe
1080 1085 1090

CAG GCC TTC AAG TAC TAC CTG GAC ATC ACC ACG CCG CCC ACG CCC CTG 3669
Gln Ala Phe Lys Tyr Tyr Leu Asp Ile Thr Thr Pro Pro Thr Pro Leu
1095 1100 1105

CAG CTG CAG CAG TTC GCC TCT CTG GCC ACT AAT GAG AAA GAG AAG CAG 3717
Gln Leu Gln Gln Phe Ala Ser Leu Ala Thr Asn Glu Lys Glu Lys Gln
1110 1115 1120

CGG TTG CTG GTC CTC AGC AAG GGG CTC CAG GAA TAT GAG GAG TGG AAG 3765
Arg Leu Leu Val Leu Ser Lys Gly Leu Gln Glu Tyr Glu Glu Trp Lys
1125 1130 1135

TGG GGC AAG AAC CCC ACA ATG GTG GAG GTG CTG GAG GAG TTC CCG TCC 3813
Trp Gly Lys Asn Pro Thr Met Val Glu Val Leu Glu Glu Phe Pro Ser
1140 1145 1150 1155

ATC CAG ATG CCG GCT ACA CTT CTC CTC ACT CAG CTG TCG CTG CTG CAG 3861
Ile Gln Met Pro Ala Thr Leu Leu Leu Thr Gln Leu Ser Leu Leu Gln
1160 1165 1170

CCT CGC TAC TAC TCC ATC AGC TCC TCT CCA GAC ATG TAC CCC GAC GAG 3909
Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Pro Asp Met Tyr Pro Asp Glu
1175 1180 1185

GTG CAC CTC ACT GTG GCC ATC GTC TCC TAC CAC ACC CGA GAC GGA GAA 3957
Val His Leu Thr Val Ala Ile Val Ser Tyr His Thr Arg Asp Gly Glu
1190 1195 1200

GGA CCA GTC CAC CAC GGG GTG TGC TCC TCC TGG CTC AAC AGA ATA CAG 4005
Gly Pro Val His His Gly Val Cys Ser Ser Trp Leu Asn Arg Ile Gln
1205 1210 1215

GCT GAC GAT GTA GTC CCC TGC TTC GTG AGA GGT GCC CCT AGC TTC CAC 4053
Ala Asp Asp Val Val Pro Cys Phe Val Arg Gly Ala Pro Ser Phe His
1220 1225 1230 1235

CTG CCT CGA AAC CCC CAG GTG CCT TGC ATC CTG GTT GGC CCA GGC ACT 4101
Leu Pro Arg Asn Pro Gln Val Pro Cys Ile Leu Val Gly Pro Gly Thr
1240 1245 1250

GGC ATC GCA CCC TTC CGA AGC TTC TGG CAA CAG CGA CAA TTT GAC ATC 4149
Gly Ile Ala Pro Phe Arg Ser Phe Trp Gln Gln Arg Gln Phe Asp Ile
1255 1260 1265

CAA CAC AAA GGA ATG AAT CCG TGC CCC ATG GTT CTG GTC TTC GGG TGT 4197
Gln His Lys Gly Met Asn Pro Cys Pro Met Val Leu Val Phe Gly Cys
1270 1275 1280

CGA CAA TCC AAG ATA GAT CAT ATC TAC AGA GAG GAG ACC CTG CAG GCT 4245
Arg Gln Ser Lys Ile Asp His Ile Tyr Arg Glu Glu Thr Leu Gln Ala
1285 1290 1295

AAG AAC AAG GGC GTC TTC AGA GAG CTG TAC ACT GCC TAT TCC CGG GAA 4293
Lys Asn Lys Gly Val Phe Arg Glu Leu Tyr Thr Ala Tyr Ser Arg Glu
1300 1305 1310 1315

CCG GAC AGG CCA AAG AAA TAT GTA CAG GAC GTG CTG CAG GAA CAG CTG 4341
Pro Asp Arg Pro Lys Lys Tyr Val Gln Asp Val Leu Gln Glu Gln Leu
1320 1325 1330

GCT AG TCT GTG TAC CGC GCC CTG AAG GAG CAA GGA GGC CAC ATT TAT 4389
Ala Glu Ser Val Tyr Arg Ala Leu Lys Glu Gln Gly Gly His Ile Tyr
1335 1340 1345

GTC TGT GGG GAC GTT ACC ATG GCC GCC GAT GTC CTC AAA GCC ATC CAG 4437
Val Cys Gly Asp Val Thr Met Ala Ala Asp Val Leu Lys Ala Ile Gln
1350 1355 1360

CGC ATA ATG ACC CAG CAG GGG AAA CTC TCA GAG GAG GAC GCT GGT GTA 4485
Arg Ile Met Thr Gln Gln Gly Lys Leu Ser Glu Glu Asp Ala Gly Val
1365 1370 1375

TTC ATC AGC AGG CTG AGG GAT GAC AAC CGG TAC CAC GAG GAC ATC TTT 4533
Phe Ile Ser Arg Leu Arg Asp Asp Asn Arg Tyr His Glu Asp Ile Phe
1380 1385 1390 1395

GGA GTC ACC CTC AGA ACG TAT GAA GTG ACC AAC CGC CTT AGA TCT GAG 4581
Gly Val Thr Leu Arg Thr Tyr Glu Val Thr Asn Arg Leu Arg Ser Glu
1400 1405 1410

TCC ATC GCC TTC ATC GAA GAG AGC AAA AAA GAC GCA GAT GAG GTT TTC 4629
Ser Ile Ala Phe Ile Glu Glu Ser Lys Lys Asp Ala Asp Glu Val Phe
1415 1420 1425

AGC TCC TAACTGGATC CTCCTGCCCC CGTGCGTGCG ATGTGGCGGC TGCCCCAAGT 4685
Ser Ser
143

GCCCAAGTAA GGGCGGCCGC AGGTTGACTA AATTCGGACA CACACGGCTG AACCGAGTGG 4745

CCCTGCTCTG CCTCTTGTCC TGTTGCTGTG TCCTGGTCCT TCTTCCTGCT CTGGGCTCTC 4805

TCAACCCCAC CCCTGGGTTT TCTCCTTGAC TCTTGGGCTA CGATGCATCA CCCTTGTACC 4865

CTGCAGTGGC TCTCACAAAA CCGCATCCTC CCCACCCCCA CCCGATTGCT GCCAAGGGCA 4925

GGTTGCGGTG CATGGCTGTT GCTCCTGTTG TTGGGGTCTG AAGGTGGCTG GCGCTGGGCC 4985

TCAGGTCACC CTGAACCAGT CCCTTGGCCA CTTAAGCCCC CTTCCACCCT CTTTTTATGA 5045

TGGTGTGTTT GT 5057

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1429 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Glu Glu Asn Thr Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile
1 5 10 15

Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val
20 25 30

Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg
35 40 45

Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile
50 55 60

Leu Ala Val Asn Asp Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala

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65                  70                  75                  80

Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile
                85                  90                  95

Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr
            100                 105                 110

Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro
        115                 120                 125

Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Ser Ala Ser Lys Asp
    130                 135                 140

Gln Ser Leu Ala Val Asp Arg Val Thr Gly Leu Gly Asn Gly Pro Gln
145                 150                 155                 160

His Ala Gln Gly His Gly Gln Gly Ala Gly Ser Val Ser Gln Ala Asn
                165                 170                 175

Gly Val Ala Ile Asp Pro Thr Met Lys Ser Thr Lys Ala Asn Leu Gln
            180                 185                 190

Asp Ile Gly Glu His Asp Glu Leu Leu Lys Glu Ile Glu Pro Val Leu
        195                 200                 205

Ser Ile Leu Asn Ser Gly Ser Lys Ala Thr Asn Arg Gly Gly Pro Ala
    210                 215                 220

Lys Ala Glu Met Lys Asp Thr Gly Ile Gln Val Asp Arg Asp Leu Asp
225                 230                 235                 240

Gly Lys Ser His Lys Ala Pro Pro Leu Gly Gly Asp Asn Asp Arg Val
                245                 250                 255

Phe Asn Asp Leu Trp Gly Lys Asp Asn Val Pro Val Ile Leu Asn Asn
            260                 265                 270

Pro Tyr Ser Glu Lys Glu Gln Ser Pro Thr Ser Gly Lys Gln Ser Pro
        275                 280                 285

Thr Lys Asn Gly Ser Pro Ser Arg Cys Pro Arg Phe Leu Lys Val Lys
    290                 295                 300

Asn Trp Glu Thr Asp Val Val Leu Thr Asp Thr Leu His Leu Lys Ser
305                 310                 315                 320

Thr Leu Glu Thr Gly Cys Thr Glu His Ile Cys Met Gly Ser Ile Met
                325                 330                 335

Leu Pro Ser Gln His Thr Arg Lys Pro Glu Asp Val Arg Thr Lys Asp
            340                 345                 350

Gln Leu Phe Pro Leu Ala Lys Glu Phe Leu Asp Gln Tyr Tyr Ser Ser
        355                 360                 365

Ile Lys Arg Phe Gly Ser Lys Ala His Met Asp Arg Leu Glu Glu Val
    370                 375                 380

Asn Lys Glu Ile Glu Ser Thr Ser Thr Tyr Gln Leu Lys Asp Thr Glu
385                 390                 395                 400

Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn Ala Ser Arg Cys Val
                405                 410                 415

Gly Arg Ile Gln Trp Ser Lys Leu Gln Val Phe Asp Ala Arg Asp Cys
            420                 425                 430
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Thr Thr Ala His Gly M t Phe Asn Tyr Ile Cys Asn His Val Lys Tyr
435 440 445

Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile Thr Ile Phe Pro Gln
450 455 460

Arg Thr Asp Gly Lys His Asp Phe Arg Val Trp Asn Ser Gln Leu Ile
465 470 475 480

Arg Tyr Ala Gly Tyr Lys Gln Pro Asp Gly Ser Thr Leu Gly Asp Pro
485 490 495

Ala Asn Val Gln Phe Thr Glu Ile Cys Ile Gln Gln Gly Trp Lys Ala
500 505 510

Pro Arg Gly Arg Phe Asp Val Leu Pro Leu Leu Leu Gln Ala Asn Gly
515 520 525

Asn Asp Pro Glu Leu Phe Gln Ile Pro Pro Glu Leu Val Leu Glu Val
530 535 540

Pro Ile Arg His Pro Lys Phe Asp Trp Phe Lys Asp Leu Gly Leu Lys
545 550 555 560

Trp Tyr Gly Leu Pro Ala Val Ser Asn Met Leu Leu Glu Ile Gly Gly
565 570 575

Leu Glu Phe Ser Ala Cys Pro Phe Ser Gly Trp Tyr Met Gly Thr Glu
580 585 590

Ile Gly Val Arg Asp Tyr Cys Asp Asn Ser Arg Tyr Asn Ile Leu Glu
595 600 605

Glu Val Ala Lys Lys Met Asp Leu Asp Met Arg Lys Thr Ser Ser Leu
610 615 620

Trp Lys Asp Gln Ala Leu Val Glu Ile Asn Ile Ala Val Leu Tyr Ser
625 630 635 640

Phe Gln Ser Asp Lys Val Thr Ile Val Asp His His Ser Ala Thr Glu
645 650 655

Ser Phe Ile Lys His Met Glu Asn Glu Tyr Arg Cys Arg Gly Gly Cys
660 665 670

Pro Ala Asp Trp Val Trp Ile Val Pro Pro Met Ser Gly Ser Ile Thr
675 680 685

Pro Val Phe His Gln Glu Met Leu Asn Tyr Arg Leu Thr Pro Ser Phe
690 695 700

Glu Tyr Gln Pro Asp Pro Trp Asn Thr His Val Trp Lys Gly Thr Asn
705 710 715 720

Gly Thr Pro Thr Lys Arg Arg Ala Ile Gly Phe Lys Lys Leu Ala Glu
725 730 735

Ala Val Lys Phe Ser Ala Lys Leu Met Gly Gln Ala Met Ala Lys Arg
740 745 750

Val Lys Ala Thr Ile Leu Tyr Ala Thr Glu Thr Gly Lys Ser Gln Ala
755 760 765

Tyr Ala Lys Thr Leu Cys Glu Ile Phe Lys His Ala Phe Asp Ala Lys
770 775 780

Ala Met Ser Met Glu Glu Tyr Asp Ile Val His Leu Glu His Glu Ala

785 790 795 800

Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn Gly Asp Pro Pro Glu
805 810 815

Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu Met Arg His Pro Asn
820 825 830

Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val Arg Phe Asn Ser Val
835 840 845

Ser Ser Tyr Ser Asp Ser Arg Lys Ser Ser Gly Asp Gly Pro Asp Leu
850 855 860

Arg Asp Asn Phe Glu Ser Thr Gly Pro Leu Ala Asn Val Arg Phe Ser
865 870 875 880

Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His Phe Cys Ala Phe Gly
885 890 895

His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly Gly Glu Arg Ile Leu
900 905 910

Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln Glu Glu Ala Phe Arg
915 920 925

Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys Asp Val Phe Cys Val
930 935 940

Gly Asp Asp Val Asn Ile Glu Lys Pro Asn Asn Ser Leu Ile Ser Asn
945 950 955 960

Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu Thr Tyr Val Ala Glu
965 970 975

Ala Pro Asp Leu Thr Gln Gly Leu Ser Asn Val His Lys Lys Arg Val
980 985 990

Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu Gln Ser Pro Lys Phe
995 1000 1005

Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr Asn Gly Asn Gln Glu
1010 1015 1020

Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val Phe Pro Gly Asn His
1025 1030 1035 1040

Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu Glu Asp Ala Pro Pro
1045 1050 1055

Ala Asn His Val Val Lys Val Glu Met Leu Glu Glu Arg Asn Thr Ala
1060 1065 1070

Leu Gly Val Ile Ser Asn Trp Lys Asp Glu Ser Arg Leu Pro Pro Cys
1075 1080 1085

Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp Ile Thr Thr Pro Pro
1090 1095 1100

Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu Ala Thr Asn Glu Lys
1105 1110 1115 1120

Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly Leu Gln Glu Tyr Glu
1125 1130 1135

Glu Trp Lys Trp Gly Lys Asn Pro Thr Met Val Glu Val Leu Glu Glu
1140 1145 1150

Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu Leu Thr Gln Leu Ser
1155 1160 1165

Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser Ser Pro Asp Met Tyr
1170 1175 1180

Pro Asp Glu Val His Leu Thr Val Ala Ile Val Ser Tyr His Thr Arg
1185 1190 1195 1200

Asp Gly Glu Gly Pro Val His His Gly Val Cys Ser Ser Trp Leu Asn
1205 1210 1215

Arg Ile Gln Ala Asp Asp Val Val Pro Cys Phe Val Arg Gly Ala Pro
1220 1225 1230

Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro Cys Ile Leu Val Gly
1235 1240 1245

Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe Trp Gln Gln Arg Gln
1250 1255 1260

Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys Pro Met Val Leu Val
1265 1270 1275 1280

Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile Tyr Arg Glu Glu Thr
1285 1290 1295

Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu Leu Tyr Thr Ala Tyr
1300 1305 1310

Ser Arg Glu Pro Asp Arg Pro Lys Lys Tyr Val Gln Asp Val Leu Gln
1315 1320 1325

Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu Lys Glu Gln Gly Gly
1330 1335 1340

His Ile Tyr Val Cys Gly Asp Val Thr Met Ala Ala Asp Val Leu Lys
1345 1350 1355 1360

Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys Leu Ser Glu Glu Asp
1365 1370 1375

Ala Gly Val Phe Ile Ser Arg Leu Arg Asp Asp Asn Arg Tyr His Glu
1380 1385 1390

Asp Ile Phe Gly Val Thr Leu Arg Thr Tyr Glu Val Thr Asn Arg Leu
1395 1400 1405

Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser Lys Lys Asp Ala Asp
1410 1415 1420

Glu Val Phe Ser Ser
1425

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5086 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: human bcl-2 cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1459..2178

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

```
GCGCCCGCCC CTCCGCGCCG CCTGCCCGCC CGCCCGCCGC GCTCCCGCCC GCCGCTCTCC      60
GTGGCCCCGC CGCGCTGCCG CCGCCGCCGC TGCCAGCGAA GGTGCCGGGG CTCCGGGCCC     120
TCCCTGCCGG CGGCCGTCAG CGCTCGGAGC GAACTGCGCG ACGGGAGGTC CGGGAGGCGA     180
CCGTAGTCGC GCCGCCGCGC AGGACCAGGA GGAGGAGAAA GGGTGCGCAG CCCGGAGGCG     240
GGGTGCGCCG GTGGGGTGCA GCGGAAGAGG GGGTCCAGGG GGGAGAACTT CGTAGCAGTC     300
ATCCTTTTTA GGAAAAGAGG GAAAAAATAA AACCCTCCCC CACCACCTCC TTCTCCCCAC     360
CCCTCGCCGC ACCACACACA GCGCGGGCTT CTAGCGCTCG GCACCGGCGG GCCAGGCGCG     420
TCCTGCCTTC ATTTATCCAG CAGCTTTTCG GAAAATGCAT TTGCTGTTCG GAGTTTAATC     480
AGAAGACGAT TCCTGCCTCC GTCCCCGGCT CCTTCATCGT CCCATCTCCC CTGTCTCTCT     540
CCTGGGGAGG CGTGAAGCGG TCCCGTGGAT AGAGATTCAT GCCTGTGTCC GCGCGTGTGT     600
GCGCGCGTAT AAATTGCCGA GAAGGGGAAA ACATCACAGG ACTTCTGCGA ATACCGGACT     660
GAAAATTGTA ATTCATCTGC CGCCGCCGCT GCCAAAAAAA AACTCGAGCT CTTGAGATCT     720
CCGGTTGGGA TTCCTGCGGA TTGACATTTC TGTGAAGCAG AAGTCTGGGA ATCGATCTGG     780
AAATCCTCCT AATTTTTACT CCCTCTCCCC CCGACTCCTG ATTCATTGGG AAGTTTCAAA     840
TCAGCTATAA CTGGAGAGTG CTGAAGATTG ATGGGATCGT TGCCTTATGC ATTTGTTTTG     900
GTTTTACAAA AAGGAAACTT GACAGAGGAT CATGCTGTAC TTAAAAAATA CAAGTAAGTC     960
TCGCACAGGA AATTGGTTTA ATGTAACTTT CAATGGAAAC CTTTGAGATT TTTTACTTAA    1020
AGTGCATTCG AGTAAATTTA ATTTCCAGGC AGCTTAATAC ATTGTTTTTA GCCGTGTTAC    1080
TTGTAGTGTG TATGCCCTGC TTTCACTCAG TGTGTACAGG GAAACGCACC TGATTTTTTA    1140
CTTATTAGTT TGTTTTTTCT TTAACCTTTC AGCATCACAG AGGAAGTAGA CTGATATTAA    1200
CAATACTTAC TAATAATAAC GTGCCTCATG AAATAAAGAT CCGAAAGGAA TTGGAATAAA    1260
AATTTCCTGC GTCTCATGCC AAGAGGGAAA CACCAGAATC AAGTGTTCCG CGTGATTGAA    1320
GACACCCCCT CGTCCAAGAA TGCAAAGCAC ATCCAATAAA ATAGCTGGAT TATAACTCCT    1380
CTTCTTTCTC TGGGGGCCGT GGGGTGGGAG CTGGGGCGAG AGGTGCCGTT GGCCCCCGTT    1440

GCTTTTCCTC TGGGAAGG ATG GCG CAC GCT GGG AGA ACG GGG TAC GAC AAC      1491
                    Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn
                     1               5                  10

CGG GAG ATA GTG ATG AAG TAC ATC CAT TAT AAG CTG TCG CAG AGG GGC      1539
Arg Glu Ile Val Met Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly
            15                  20                  25
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TAC GAG TGG GAT GCG GGA GAT GTG GGC GCC GCG CCC CCG GGG GCC GCC 1587
Tyr Glu Trp Asp Ala Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala
30 35 40

CCC GCA CCG GGC ATC TTC TCC TCC CAG CCC GGG CAC ACG CCC CAT CCA 1635
Pro Ala Pro Gly Ile Phe Ser Ser Gln Pro Gly His Thr Pro His Pro
45 50 55

GCC GCA TCC CGC GAC CCG GTC GCC AGG ACC TCG CCG CTG CAG ACC CCG 1683
Ala Ala Ser Arg Asp Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro
60 65 70 75

GCT GCC CCC GGC GCC GCC GCG GGG CCT GCG CTC AGC CCG GTG CCA CCT 1731
Ala Ala Pro Gly Ala Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro
80 85 90

GTG GTC CAC CTG GCC CTC CGC CAA GCC GGC GAC GAC TTC TCC CGC CGC 1779
Val Val His Leu Ala Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg
95 100 105

TAC CGC GGC GAC TTC GCC GAG ATG TCC AGC CAG CTG CAC CTG ACG CCC 1827
Tyr Arg Gly Asp Phe Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro
110 115 120

TTC ACC GCG CGG GGA CGC TTT GCC ACG GTG GTG GAG GAG CTC TTC AGG 1875
Phe Thr Ala Arg Gly Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg
125 130 135

GAC GGG GTG AAC TGG GGG AGG ATT GTG GCC TTC TTT GAG TTC GGT GGG 1923
Asp Gly Val Asn Trp Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly
140 145 150 155

GTC ATG TGT GTG GAG AGC GTC AAC CGG GAG ATG TCG CCC CTG GTG GAC 1971
Val Met Cys Val Glu Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp
160 165 170

AAC ATC GCC CTG TGG ATG ACT GAG TAC CTG AAC CGG CAC CTG CAC ACC 2019
Asn Ile Ala Leu Trp Met Thr Glu Tyr Leu Asn Arg His Leu His Thr
175 180 185

TGG ATC CAG GAT AAC GGA GGC TGG GAT GCC TTT GTG GAA CTG TAC GGC 2067
Trp Ile Gln Asp Asn Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly
190 195 200

CCC AGC ATG CGG CCT CTG TTT GAT TTC TCC TGG CTG TCT CTG AAG ACT 2115
Pro Ser Met Arg Pro Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr
205 210 215

CTG CTC AGT TTG GCC CTG GTG GGA GCT TGC ATC ACC CTG GGT GCC TAT 2163
Leu Leu Ser Leu Ala Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr
220 225 230 235

CTG AGC CAC AAG TGAAGTCAAC ATGCCTGCCC CAAACAAATA TGCAAAAGGT 2215
Leu Ser His Lys
240

TCACTAAAGC AGTAGAAATA ATATGCATTG TCAGTGATGT ACCATGAAAC AAAGCTGCAG 2275

GCTGTTTAAG AAAAAATAAC ACACATATAA ACATCACACA CACAGACAGA CACACACACA 2335

CACAACAATT AACAGTCTTC AGGCAAAACG TCGAATCAGC TATTTACTGC CAAAGGGAAA 2395

TATCATTTAT TTTTTACATT ATTAAGAAAA AAGATTTATT TATTTAAGAC AGTCCCATCA 2455

AAACTCCGTC TTTGGAAATC CGACCACTAA TTGCCAAACA CCGCTTCGTG TGGCTCCACC 2515

TGGATGTTCT GTGCCTGTAA ACATAGATTC GCTTTCCATG TTGTTGGCCG GATCACCATC 2575

TGAAGAGCAG ACGGATGGAA AAAGGACCTG ATCATTGGGG AAGCTGGCTT TCTGGCTGCT 2635
GGAGGCTGGG GAGAAGGTGT TCATTCACTT GCATTTCTTT GCCCTGGGGG CGTGATATTA 2695
ACAGAGGGAG GGTTCCCGTG GGGGGAAGTC CATGCCTCCC TGGCCTGAAG AAGAGACTCT 2755
TTGCATATGA CTCACATGAT GCATACCTGG TGGGAGGAAA AGAGTTGGGA ACTTCAGATG 2815
GACCTAGTAC CCACTGAGAT TTCCACGCCG AAGGACAGCG ATGGGAAAAA TGCCCTTAAA 2875
TCATAGGAAA GTATTTTTTT AAGCTACCAA TTGTGCCGAG AAAAGCATTT TAGCAATTTA 2935
TACAATATCA TCCAGTACCT TAAACCCTGA TTGTGTATAT TCATATATTT TGGATACGCA 2995
CCCCCCAACT CCCAATACTG GCTCTGTCTG AGTAAGAAAC AGAATCCTCT GGAACTTGAG 3055
GAAGTGAACA TTTCGGTGAC TTCCGATCAG GAAGGCTAGA GTTACCCAGA GCATCAGGCC 3115
GCCACAAGTG CCTGCTTTTA GGAGACCGAA GTCCGCAGAA CCTACCTGTG TCCCAGCTTG 3175
GAGGCCTGGT CCTGGAACTG AGCCGGGCCC TCACTGGCCT CCTCCAGGGA TGATCAACAG 3235
GGTAGTGTGG TCTCCGAATG TCTGGAAGCT GATGGATGGA GCTCAGAATT CCACTGTCAA 3295
GAAAGAGCAG TAGAGGGGTG TGGCTGGGCC TGTCACCCTG GGGCCCTCCA GGTAGGCCCG 3355
TTTTCACGTG GAGCATAGGA GCCACGACCC TTCTTAAGAC ATGTATCACT GTAGAGGGAA 3415
GGAACAGAGG CCCTGGGCCT TCCTATCAGA AGGACATGGT GAAGGCTGGG AACGTGAGGA 3475
GAGGCAATGG CCACGGCCCA TTTTGGCTGT AGCACATGGC ACGTTGGCTG TGTGGCCTTG 3535
GCCACCTGTG AGTTTAAAGC AAGGCTTTAA ATGACTTTGG AGAGGGTCAC AAATCCTAAA 3595
AGAAGCATTG AAGTGAGGTG TCATGGATTA ATTGACCCCT GTCTATGGAA TTACATGTAA 3655
AACATTATCT TGTCACTGTA GTTTGGTTTT ATTTGAAAAC CTGACAAAAA AAAAGTTCCA 3715
GGTGTGGAAT ATGGGGGTTA TCTGTACATC CTGGGGCATT AAAAAAAAAT CAATGGTGGG 3775
GAACTATAAA GAAGTAACAA AAGAAGTGAC ATCTTCAGCA AATAAACTAG GAAATTTTTT 3835
TTTCTTCCAG TTTAGAATCA GCCTTGAAAC ATTGATGGAA TAACTCTGTG GCATTATTGC 3895
ATTATATACC ATTTATCTGT ATTAACTTTG GAATGTACTC TGTTCAATGT TTAATGCTGT 3955
GGTTGATATT TCGAAAGCTG CTTTAAAAAA ATACATGCAT CTCAGCGTTT TTTTGTTTTT 4015
AATTGTATTT AGTTATGGCC TATACACTAT TTGTGAGCAA AGGTGATCGT TTTCTGTTTG 4075
AGATTTTTAT CTCTTGATTC TTCAAAAGCA TTCTGAGAAG GTGAGATAAG CCCTGAGTCT 4135
CAGCTACCTA AGAAAAACCT GGATGTCACT GGCCACTGAG GAGCTTTGTT TCAACCAAGT 4195
CATGTGCATT TCCACGTCAA CAGAATTGTT TATTGTGACA GTTATATCTG TTGTCCCTTT 4255
GACCTTGTTT CTTGAAGGTT TCCTCGTCCC TGGGCAATTC CGCATTTAAT TCATGGTATT 4315
CAGGATTACA TGCATGTTTG GTTAAACCCA TGAGATTCAT TCAGTTAAAA ATCCAGATGG 4375
CGAATGACCA GCAGATTCAA ATCTATGGTG GTTTGACCTT TAGAGAGTTG CTTTACGTGG 4435
CCTGTTTCAA CACAGACCCA CCCAGAGCCC TCCTGCCCTC CTTCCGCGGG GGCTTTCTCA 4495
TGGCTGTCCT TCAGGGTCTT CCTGAAATGC AGTGGTCGTT ACGCTCCACC AAGAAAGCAG 4555
GAAACCTGTG GTATGAAGCC AGACCTCCCC GGCGGGCCTC AGGGAACAGA ATGATCAGAC 4615

CTTTGAATGA TTCTAATTTT TAAGCAAAAT ATTATTTTAT GAAAGGTTTA CATTGTCAAA 4675

GTGATGAATA TGGAATATCC AATCCTGTGC TGCTATCCTG CCAAAATCAT TTTAATGGAG 4735

TCAGTTTGCA GTATGCTCCA CGTGGTAAGA TCCTCCAAGC TGCTTTAGAA GTAACAATGA 4795

AGAACGTGGA CGTTTTTAAT ATAAAGCCTG TTTTGTCTTT TGTTGTTGTT CAAACGGGAT 4855

TCACAGAGTA TTTGAAAAAT GTATATATAT TAAGAGGTCA CGGGGGCTAA TTGCTAGCTG 4915

GCTGCCTTTT GCTGTGGGGT TTTGTTACCT GGTTTTAATA ACAGTAAATG TGCCCAGCCT 4975

CTTGGCCCCA GAACTGTACA GTATTGTGGC TGCACTTGCT CTAAGAGTAG TTGATGTTGC 5035

ATTTTCCTTA TTGTTAAAAA CATGTTAGAA GCAATGAATG TATATAAAAG C 5086

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ala His Ala Gly Arg Thr Gly Tyr Asp Asn Arg Glu Ile Val Met
1 5 10 15

Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala
20 25 30

Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Ile
35 40 45

Phe Ser Ser Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp
50 55 60

Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala
65 70 75 80

Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Ala
85 90 95

Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Gly Asp Phe
100 105 110

Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly
115 120 125

Arg Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp
130 135 140

Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu
145 150 155 160

Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp
165 170 175

Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn
180 185 190

Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro
195 200 205

```
Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala
    210                 215                 220

Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Ser His Lys
225                 230                 235
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(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1846 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: bcl-2 fusion gene; Seto, et al., EMBO J 7:123 (1988)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 887..1606

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

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ACCACCTCCT TCTCCCCACC CCTCGCCGCA CCACACACAG CGCGGGCTTC TGGCGCTCGG      60

CACCGGCGGG CCAGGCGCGT CCTGTCTTCA TTTATCCAGC AGCTTTTCGG AAAATCCATT     120

TGGTGTTCGG AGTTTAATCA GAAGAGGATT CCTGCCTCCG TCCCCGGCTC CTTCATCGTC     180

CCCTCTCCCC TGTCTCTCTC CTGGGGAGGC GTGAAGAGAG ATTCATGCCT GTGCCCGCGC     240

GTGTGTGCGC GCGTATAAAT TGCCGAGAAG GGGAAAACAT CACAGGACTT CTGCGAATAC     300

CGGACTGAAA ATTGTAGCTC ATCTGCCGCC GCCGCTGCCT TTTTTTTTTC TCGAGCTCTT     360

GAGATCTCCG GTTGGGACTC CTGCGGATTG ACATTTCTGT GAAGCAGAAG TCTGGGAATC     420

GATCTGGAAA TCCTCCTAAT TTTTACTCCC TCTCCCCCCG ACTCCTGATT CATTGGGAAG     480

TTTCAAATCA GCTATAACTG GAGAGAGCTG AAGATTGATG GGATCGTTGC CTTATGCCTT     540

TGTTTTGGTT TTACAAAAAG GAAACTTGAC AGAGGATCAT GCTATACTTA AAAAATACAA     600

CATCGCAGAG GAAGTAGACT CATATTAAAA ATACTTACTA ATAATAACGT GCCTCATGAA     660

GTAAAGATCC GAAAGGAATT GGAATAAAAC TTTCCTGCAT CTCAAGCCAA GGGGGAAACA     720

CCAGAATCAA GTGTTCCGCG TGATTGAAGA CACCCCCTCG TCCAAGAATG CAAAGCACAT     780

CCAATAAAAG AGCTGGATTA TAACTCCTCT TCTTTCTCTG GGGGCCGTGG GGTAGGGGCT     840

GGGGCGAGAG GTGCCGTTGG CCCCCGTTGC TTTTCCTCTG GGAGGG ATG GCG CAC       895
                                                   Met Ala His
                                                     1

GCT GGG AGA AGT GGT TAC GAT AAC CGG GAG ATA GTG ATG AAG TAC ATC      943
Ala Gly Arg Ser Gly Tyr Asp Asn Arg Glu Ile Val Met Lys Tyr Ile
    5                   10                  15
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CAT TAT AAG CTG TCG CAG AGG GGC TAC GAG TGG GAT GCG GGA GAT GTG 991
His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala Gly Asp Val
20 25 30 35

GGC GCC GCG CCC CCG GGG GCC GCC CCC GCA CCG GGC TTC TTC TCC TCC 1039
Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Phe Phe Ser Ser
40 45 50

CAG CCC GGG CAC ACG CCC CAT CCA GCC GCA TCC CGG GAC CCG GTC GCC 1087
Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp Pro Val Ala
55 60 65

AGG ACC TCG CCA CTA CAG ACC CCG GCT GCC CCC GGC GCC GCC GCG GGG 1135
Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala Ala Ala Gly
70 75 80

CCT GCG CTC AGC CCG GTG CCA CCT GTG GTC CAC CTG ACC CTC CGC CAG 1183
Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Thr Leu Arg Gln
85 90 95

GCC GGC GAC GAC TTC TCC CGC CGC TAC CGC CGC GAC TTC GCC GAG ATG 1231
Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Arg Asp Phe Ala Glu Met
100 105 110 115

TCC AGC CAG CTG CAC CTG ACG CCC TTC ACC GCG CGG GGA TGC TTT GCC 1279
Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly Cys Phe Ala
120 125 130

ACG GTG GTG GAG GAG CTC TTC AGG GAC GGG GTG AAC TGG GGG AGG ATT 1327
Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp Gly Arg Ile
135 140 145

GTG GCC TTC TTT GAG TTC GGT GGG GTC ATG TGT GTG GAG AGC GTC AAC 1375
Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu Ser Val Asn
150 155 160

CGG GAG ATG TCG CCC CTG GTG GAC AAC ATC GCC CTG TGG ATG ACT GAG 1423
Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp Met Thr Glu
165 170 175

TAC CTG AAC CGG CAC CTG CAC ACC TGG ATC CAG GAT AAC GGA GGC TGG 1471
Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn Gly Gly Trp
180 185 190 195

GAT GCC TTT GTG GAA CTG TAC GGC CCC AGC ATG CGG CCT CTG TTT GAT 1519
Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro Leu Phe Asp
200 205 210

TTC TCC TGG CTG TCT CTG AAG ACT CTG CTC AGT TTG GCC CTG GTG GGA 1567
Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala Leu Val Gly
215 220 225

GCT TGC ATC ACC CTG GGT GCC TAT CTG GGC CAC AAG TGAAGTCAAC 1613
Ala Cys Ile Thr Leu Gly Ala Tyr Leu Gly His Lys
230 235 240

ATGCCTGCCC CAAACAAATA TGCAAAAGGT TCACTAAAGC AGTAGAAATA ATATGCATTG 1673

TCAGTGATGT ACCATGAAAC AAAGCTGCAG GCTGTTTAAG AAAAAATAAC ACACATATAA 1733

ACATCACACA CACAGACAGA CACACACACA CACAACAATT AACAGTCTTC AGGCAAAACG 1793

TCGAATCAGC TATTTACTGC CAAAGGGAAA TATCATTTAT TTTTTACATT ATT 1846

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

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Met Ala His Ala Gly Arg Ser Gly Tyr Asp Asn Arg Glu Ile Val Met
  1               5                  10                  15

Lys Tyr Ile His Tyr Lys Leu Ser Gln Arg Gly Tyr Glu Trp Asp Ala
             20                  25                  30

Gly Asp Val Gly Ala Ala Pro Pro Gly Ala Ala Pro Ala Pro Gly Phe
         35                  40                  45

Phe Ser Ser Gln Pro Gly His Thr Pro His Pro Ala Ala Ser Arg Asp
     50                  55                  60

Pro Val Ala Arg Thr Ser Pro Leu Gln Thr Pro Ala Ala Pro Gly Ala
 65                  70                  75                  80

Ala Ala Gly Pro Ala Leu Ser Pro Val Pro Pro Val Val His Leu Thr
                 85                  90                  95

Leu Arg Gln Ala Gly Asp Asp Phe Ser Arg Arg Tyr Arg Arg Asp Phe
            100                 105                 110

Ala Glu Met Ser Ser Gln Leu His Leu Thr Pro Phe Thr Ala Arg Gly
        115                 120                 125

Cys Phe Ala Thr Val Val Glu Glu Leu Phe Arg Asp Gly Val Asn Trp
    130                 135                 140

Gly Arg Ile Val Ala Phe Phe Glu Phe Gly Gly Val Met Cys Val Glu
145                 150                 155                 160

Ser Val Asn Arg Glu Met Ser Pro Leu Val Asp Asn Ile Ala Leu Trp
                165                 170                 175

Met Thr Glu Tyr Leu Asn Arg His Leu His Thr Trp Ile Gln Asp Asn
            180                 185                 190

Gly Gly Trp Asp Ala Phe Val Glu Leu Tyr Gly Pro Ser Met Arg Pro
        195                 200                 205

Leu Phe Asp Phe Ser Trp Leu Ser Leu Lys Thr Leu Leu Ser Leu Ala
    210                 215                 220

Leu Val Gly Ala Cys Ile Thr Leu Gly Ala Tyr Leu Gly His Lys
225                 230                 235
```

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4353 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Human NOS-1 gene, Fujisawa, et al, J. Neurochem 63:140 1994

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..4305

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

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ATG GAG GAT CAC ATG TTC GGT GTT CAG CAA ATC CAG CCC AAT GTC ATT      48
Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile
  1               5                  10                  15

TCT GTT CGT CTC TTC AAG CGC AAA GTT GGG GGC CTG GGA TTT CTG GTG      96
Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val
             20                  25                  30

AAG GAG CGG GTC AGT AAG CCG CCC GTG ATC ATC TCT GAC CTG ATT CGT     144
Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg
         35                  40                  45

GGG GGC GCC GCA GAG CAG AGT GGC CTC ATC CAG GCC GGA GAC ATC ATT     192
Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile
     50                  55                  60

CTT GCG GTC AAC GGC CGG CCC TTG GTG GAC CTG AGC TAT GAC AGC GCC     240
Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala
 65                  70                  75                  80

CTG GAG GTA CTC AGA GGC ATT GCC TCT GAG ACC CAC GTG GTC CTC ATT     288
Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile
                 85                  90                  95

CTG AGG GGC CCT GAA GGT TTC ACC ACG CAC CTG GAG ACC ACC TTT ACA     336
Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr
            100                 105                 110

GGT GAT GGG ACC CCC AAG ACC ATC CGG GTG ACA CAG CCC CTG GGT CCC     384
Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro
        115                 120                 125

CCC ACC AAA GCC GTG GAT CTG TCC CAC CAG CCA CCG GCC GGC AAA GAA     432
Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala Gly Lys Glu
    130                 135                 140

CAG CCC CTG GCA GTG GAT GGG GCC TCG GGT CCC GGG AAT GGG CCT CAG     480
Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn Gly Pro Gln
145                 150                 155                 160

CAT GCC TAC GAT GAT GGG CAG GAG GCT GGC TCA CTC CCC CAT GCC AAC     528
His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro His Ala Asn
                165                 170                 175

GGC CTG GCC CCC AGG CCC CCA GGC CAG GAC CCC GCG AAG AAA GCA ACC     576
Gly Leu Ala Pro Arg Pro Pro Gly Gln Asp Pro Ala Lys Lys Ala Thr
            180                 185                 190

AGA GTC AGC CTC CAA GGC AGA GGG GAG AAC AAT GAA CTG CTC AAG GAG     624
Arg Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu Lys Glu
        195                 200                 205

ATA GAG CCT GTG CTG AGC CTT CTC ACC AGT GGG AGC AGA GGG GTC AAG     672
Ile Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly Val Lys
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    210                      215                      220

GGA GGG GCA CCT GCC AAG GCA GAG ATG AAA GAT ATG GGA ATC CAG GTG      720
Gly Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile Gln Val
225                 230                      235                 240

GAC AGA GAT TTG GAC GGC AAG TCA CAC AAA CCT CTG CCC CTC GGC GTG      768
Asp Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu Gly Val
                245                 250                      255

GAG AAC GAC CGA GTC TTC AAT GAC CTA TGG GGG AAG GGC AAT GTG CCT      816
Glu Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn Val Pro
            260                 265                      270

GTC GTC CTC AAC AAC CCA TAT TCA GAG AAG GAG CAG CCC CCC ACC TCA      864
Val Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Pro Thr Ser
        275                 280                      285

GGA AAA CAG TCC CCC ACA AAG AAT GGC AGC CCC TCC AAG TGT CCA CGC      912
Gly Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Ser Lys Cys Pro Arg
    290                 295                      300

TTC CTC AAG GTC AAG AAC TGG GAG ACT GAG GTG GTT CTC ACT GAC ACC      960
Phe Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr Asp Thr
305                 310                      315                 320

CTC CAC CTT AAG AGC ACA TTG GAA ACG GGA TGC ACT GAG TAC ATC TGC     1008
Leu His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr Ile Cys
                325                 330                      335

ATG GGC TCC ATC ATG CAT CCT TCT CAG CAT GCA AGG AGG CCT GAA GAC     1056
Met Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro Glu Asp
            340                 345                      350

GTC CGC ACA AAA GGA CAG CTC TTC CCT CTC GCC AAA GAG TTT ATT GAT     1104
Val Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe Ile Asp
        355                 360                      365

CAA TAC TAT TCA TCA ATT AAA AGA TTT GGC TCC AAA GCC CAC ATG GAA     1152
Gln Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His Met Glu
    370                 375                      380

AGG CTG GAA GAG GTG AAC AAA GAG ATC GAC ACC ACT AGC ACT TAC CAG     1200
Arg Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr Tyr Gln
385                 390                      395                 400

CTC AAG GAC ACA GAG CTC ATC TAT GGG GCC AAG CAC GCC TGG CGG AAT     1248
Leu Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn
                405                 410                      415

GCC TCG CGC TGT GTG GGC AGG ATC CAG TGG TCC AAG CTG CAG GTA TTC     1296
Ala Ser Arg Cys Val Gly Arg Ile Gln Trp Ser Lys Leu Gln Val Phe
            420                 425                      430

GAT GCC CGT GAC TGC ACC ACG GCC CAC GGG ATG TTC AAC TAC ATC TGT     1344
Asp Ala Arg Asp Cys Thr Thr Ala His Gly Met Phe Asn Tyr Ile Cys
        435                 440                      445

AAC CAT GTC AAG TAT GCC ACC AAC AAA GGG AAC CTC AGG TCT GCC ATC     1392
Asn His Val Lys Tyr Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile
    450                 455                      460

ACC ATA TTC CCC CAG AGG ACA GAC GGC AAG CAC GAC TTC CGA GTC TGG     1440
Thr Ile Phe Pro Gln Arg Thr Asp Gly Lys His Asp Phe Arg Val Trp
465                 470                      475                 480

AAC TCC CAG CTC ATC CGC TAC GCT GGC TAC AAG CAG CCT GAC GGC TCC     1488
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Asn Ser Gln Leu Ile Arg Tyr Ala Gly Tyr Lys Gln Pro Asp Gly Ser
                485                 490                 495

ACC CTG GGG GAC CCA GCC AAT GTG CAG TTC ACA GAG ATA TGC ATA CAG       1536
Thr Leu Gly Asp Pro Ala Asn Val Gln Phe Thr Glu Ile Cys Ile Gln
            500                 505                 510

CAG GGC TGG AAA CCG CCT AGA GGC CGC TTC GAT GTC CTG CCG CTC CTG       1584
Gln Gly Trp Lys Pro Pro Arg Gly Arg Phe Asp Val Leu Pro Leu Leu
        515                 520                 525

CTT CAG GCC AAC GGC AAT GAC CCT GAG CTC TTC CAG ATT CCT CCA GAG       1632
Leu Gln Ala Asn Gly Asn Asp Pro Glu Leu Phe Gln Ile Pro Pro Glu
    530                 535                 540

CTG GTG TTG GAA GTT CCC ATC AGG CAC CCC AAG TTT GAG TGG TTC AAG       1680
Leu Val Leu Glu Val Pro Ile Arg His Pro Lys Phe Glu Trp Phe Lys
545                 550                 555                 560

GAC CTG GGG CTG AAG TGG TAC GGC CTC CCC GCC GTG TCC AAC ATG CTC       1728
Asp Leu Gly Leu Lys Trp Tyr Gly Leu Pro Ala Val Ser Asn Met Leu
                565                 570                 575

CTA GAG ATT GGC GGC CTG GAG TTC AGC GCC TGT CCC TTC AGT GGC TGG       1776
Leu Glu Ile Gly Gly Leu Glu Phe Ser Ala Cys Pro Phe Ser Gly Trp
            580                 585                 590

TAC ATG GGC ACA GAG ATT GGT GTC CGC GAC TAC TGT GAC AAC TCC CGC       1824
Tyr Met Gly Thr Glu Ile Gly Val Arg Asp Tyr Cys Asp Asn Ser Arg
        595                 600                 605

TAC AAT ATC CTG GAG GAA GTG GCC AAG AAG ATG AAC TTA GAC ATG AGG       1872
Tyr Asn Ile Leu Glu Glu Val Ala Lys Lys Met Asn Leu Asp Met Arg
    610                 615                 620

AAG ACG TCC TCC CTG TGG AAG GAC CAG GCG CTG GTG GAG ATC AAT ATC       1920
Lys Thr Ser Ser Leu Trp Lys Asp Gln Ala Leu Val Glu Ile Asn Ile
625                 630                 635                 640

GCG GTT CTC TAT AGC TTC CAG AGT GAC AAA GTG ACC ATT GTT GAC CAT       1968
Ala Val Leu Tyr Ser Phe Gln Ser Asp Lys Val Thr Ile Val Asp His
                645                 650                 655

CAC TCC GCC ACC GAG TCC TTC ATT AAG CAC ATG GAG AAT GAG TAC CGC       2016
His Ser Ala Thr Glu Ser Phe Ile Lys His Met Glu Asn Glu Tyr Arg
            660                 665                 670

TGC CGG GGG GGC TGC CCT GCC GAC TGG GTG TGG ATC GTG CCC CCC ATG       2064
Cys Arg Gly Gly Cys Pro Ala Asp Trp Val Trp Ile Val Pro Pro Met
        675                 680                 685

TCC GGA AGC ATC ACC CCT GTG TTC CAC CAG GAG ATG CTC AAC TAC CGG       2112
Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met Leu Asn Tyr Arg
    690                 695                 700

CTC ACC CCC TCC TTC GAA TAC CAG CCT GAT CCC TGG AAC ACG CAT GTC       2160
Leu Thr Pro Ser Phe Glu Tyr Gln Pro Asp Pro Trp Asn Thr His Val
705                 710                 715                 720

TGG AAA GGC ACC AAC GGG ACC CCC ACA AAG CGG CGA GCC ATC GGC TTC       2208
Trp Lys Gly Thr Asn Gly Thr Pro Thr Lys Arg Arg Ala Ile Gly Phe
                725                 730                 735

AAG AAG CTA GCA GAA GCT GTC AAG TTC TCG GCC AAG CTG ATG GGG CAG       2256
Lys Lys Leu Ala Glu Ala Val Lys Phe Ser Ala Lys Leu Met Gly Gln
            740                 745                 750
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GCT ATG GCC AAG AGG GTG AAA GCG ACC ATC CTC TAT GCC ACA GAG ACA 2304
Ala Met Ala Lys Arg Val Lys Ala Thr Ile Leu Tyr Ala Thr Glu Thr
755 760 765

GGC AAA TCG CAA GCT TAT GCC AAG ACC TTG TGT GAG ATC TTC AAA CAC 2352
Gly Lys Ser Gln Ala Tyr Ala Lys Thr Leu Cys Glu Ile Phe Lys His
770 775 780

GCC TTT GAT GCC AAG GTG ATG TCC ATG GAA GAA TAT GAC ATT GTG CAC 2400
Ala Phe Asp Ala Lys Val Met Ser Met Glu Glu Tyr Asp Ile Val His
785 790 795 800

CTG GAA CAT GAA ACT CTG GTC CTT GTG GTC ACC AGC ACC TTT GGC AAT 2448
Leu Glu His Glu Thr Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn
805 810 815

GGA GAT CCC CCT GAG AAT GGG GAG AAA TTC GGC TGT GCT TTG ATG GAA 2496
Gly Asp Pro Pro Glu Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu
820 825 830

ATG AGG CAC CCC AAC TCT GTG CAG GAA GAA AGG AAG AGC TAC AAG GTC 2544
Met Arg His Pro Asn Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val
835 840 845

CGA TTC AAC AGC GTC TCC TCC TAC TCT GAC TCC CAA AAA TCA TCA GGC 2592
Arg Phe Asn Ser Val Ser Ser Tyr Ser Asp Ser Gln Lys Ser Ser Gly
850 855 860

GAT GGG CCC GAC CTC AGA GAC AAC TTT GAG AGT GCT GGA CCC CTG GCC 2640
Asp Gly Pro Asp Leu Arg Asp Asn Phe Glu Ser Ala Gly Pro Leu Ala
865 870 875 880

AAT GTG AGG TTC TCA GTT TTT GGC CTC GGC TCA CGA GCA TAC CCT CAC 2688
Asn Val Arg Phe Ser Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His
885 890 895

TTT TGC GCC TTC GGA CAC GCT GTG GAC ACC CTC CTG GAA GAA CTG GGA 2736
Phe Cys Ala Phe Gly His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly
900 905 910

GGG GAG AGG ATC CTG AAG ATG AGG GAA GGG GAT GAG CTC TGT GGG CAG 2784
Gly Glu Arg Ile Leu Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln
915 920 925

GAA GAG GCT TTC AGG ACC TGG GCC AAG AAG GTC TTC AAG GCA GCC TGT 2832
Glu Glu Ala Phe Arg Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys
930 935 940

GAT GTC TTC TGT GTG GGA GAT GAT GTC AAC ATT GAA AAG GCC AAC AAT 2880
Asp Val Phe Cys Val Gly Asp Asp Val Asn Ile Glu Lys Ala Asn Asn
945 950 955 960

TCC CTC ATC AGC AAT GAT CGC AGC TGG AAG AGA AAC AAG TTC CGC CTC 2928
Ser Leu Ile Ser Asn Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu
965 970 975

ACC TTT GTG GCC GAA GCT CCA GAA CTC ACA CAA GGT CTA TCC AAT GTC 2976
Thr Phe Val Ala Glu Ala Pro Glu Leu Thr Gln Gly Leu Ser Asn Val
980 985 990

CAC AAA AAG CGA GTC TCA GCT GCC CGG CTC CTT AGC CGT CAA AAC CTC 3024
His Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu
995 1000 1005

CAG AGC CCT AAA TCC AGT CGG TCA ACT ATC TTC GTG CGT CTC CAC ACC 3072
Gln Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr
1010 1015 1020

AAC GGG AGC CAG GAG CTG CAG TAC CAG CCT GGG GAC CAC CTG GGT GTC 3120
Asn Gly Ser Gln Glu Leu ln Tyr Gln Pro Gly Asp His Leu Gly Val
1025 1030 1035 1040

TTC CCT GGC AAC CAC GAG GAC CTC GTG AAT GCC CTG ATC GAG CGG CTG 3168
Phe Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu
1045 1050 1055

GAG GAC GCG CCG CCT GTC AAC CAG ATG GTG AAA GTG GAA CTG CTG GAG 3216
Glu Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu Leu Glu
1060 1065 1070

GAG CGG AAC ACG GCT TTA GGT GTC ATC AGT AAC TGG ACA GAC GAG CTC 3264
Glu Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp Glu Leu
1075 1080 1085

CGC CTC CCA CCC TGC ACC ATC TTC CAG GCC TTC AAG TAC TAC CTG GAC 3312
Arg Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp
1090 1095 1100

ATC ACC ACG CCA CCA ACG CCC CTG CAG CTG CAG CAG TTT GCC TCC CTA 3360
Ile Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu
1105 1110 1115 1120

GCT ACC AGC GAG AAG GAG AAG CAG CGT CTG CTG GTC CTC AGC AAG GGT 3408
Ala Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly
1125 1130 1135

TTG CAG GAG TAC GAG GAA TGG AAA TGG GGC AAG AAC CCC ACC ATC GTG 3456
Leu Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr Ile Val
1140 1145 1150

GAG GTG CTG GAG GAG TTC CCA TCT ATC CAG ATG CCG GCC ACC CTG CTC 3504
Glu Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu
1155 1160 1165

CTG ACC CAG CTG TCC CTG CTG CAG CCC CGC TAC TAT TCC ATC AGC TCC 3552
Leu Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser
1170 1175 1180

TCC CCA GAC ATG TAC CCT GAT GAA GTG CAC CTC ACT GTG GCC ATC GTT 3600
Ser Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala Ile Val
1185 1190 1195 1200

TCC TAC CGC ACT CGA GAT GGA GAA GGA CCA ATT CAC CAC GGC GTA TGC 3648
Ser Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly Val Cys
1205 1210 1215

TCC TCC TGG CTC AAC CGG ATA CAG GCT GAC GAA CTG GTC CCC TGT TTC 3696
Ser Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro Cys Phe
1220 1225 1230

GTG AGA GGA GCA CCC AGC TTC CAC CTG CCC CGG AAC CCC CAA GTC CCC 3744
Val Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro
1235 1240 1245

TGC ATC CTC GTT GGA CCA GGC ACC GGC ATT GCC CCT TTC CGA AGC TTC 3792
Cys Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe
1250 1255 1260

TGG CAA CAG CGG CAA TTT GAT ATC CAA CAC AAA GGA ATG AAC CCC TGC 3840
Trp Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys
1265 1270 1275 1280

CCC ATG GTC CTG GTC TTC GGG TGC CGG CAA TCC AAG ATA GAT CAT ATC 3888
Pro Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile
1285 1290 1295

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TAC AGG GAA GAG ACC CTG CAG GCC AAG AAC AAG GGG GTC TTC AGA GAG        3936
Tyr Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu
            1300                1305                1310

CTG TAC ACG GCT TAC TCC CGG GAG CCA GAC AAA CCA AAG AAG TAC GTG        3984
Leu Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys Tyr Val
        1315                1320                1325

CAG GAC ATC CTG CAG GAG CAG CTG GCG GAG TCT GTG TAC CGA GCC CTG        4032
Gln Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu
    1330                1335                1340

AAG GAG CAA GGG GGC CAC ATA TAC GTC TGT GGG GAC GTC ACC ATG GCT        4080
Lys Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr Met Ala
1345                1350                1355                1360

GCT GAT GTC CTC AAA GCC ATC CAG CGC ATC ATG ACC CAG CAG GGG AAG        4128
Ala Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys
                1365                1370                1375

CTC TCG GCA GAG GAC GCC GGC GTA TTC ATC AGC CGG ATG AGG GAT GAC        4176
Leu Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg Asp Asp
            1380                1385                1390

AAC CGA TAC CAT GAG GAT ATT TTT GGA GTC ACC CTG CGA ACG TAC GAA        4224
Asn Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr Tyr Glu
        1395                1400                1405

GTG ACC AAC CGC CTT AGA TCT GAG TCC ATT GCC TTC ATT GAA GAG AGC        4272
Val Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser
    1410                1415                1420

AAA AAA GAC ACC GAT GAG GTT TTC AGC TCC TAACTGGACC CTCTTGCCCA          4322
Lys Lys Asp Thr Asp Glu Val Phe Ser Ser
1425                1430                143

GCCGGCTGCA AGTTTGTAAG CGCGGACAG A                                       4353
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(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1434 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

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Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile
  1               5                  10                  15

Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val
             20                  25                  30

Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg
         35                  40                  45

Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile
     50                  55                  60

Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala
 65                  70                  75                  80

Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile
                 85                  90                  95
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Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr
100 105 110

Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro
115 120 125

Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala Gly Lys Glu
130 135 140

Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn Gly Pro Gln
145 150 155 160

His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro His Ala Asn
165 170 175

Gly Leu Ala Pro Arg Pro Pro Gly Gln Asp Pro Ala Lys Lys Ala Thr
180 185 190

Arg Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu Lys Glu
195 200 205

Ile Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly Val Lys
210 215 220

Gly Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile Gln Val
225 230 235 240

Asp Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu Gly Val
245 250 255

Glu Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn Val Pro
260 265 270

Val Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Pro Thr Ser
275 280 285

Gly Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Ser Lys Cys Pro Arg
290 295 300

Phe Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr Asp Thr
305 310 315 320

Leu His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr Ile Cys
325 330 335

Met Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro Glu Asp
340 345 350

Val Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe Ile Asp
355 360 365

Gln Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His Met Glu
370 375 380

Arg Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr Tyr Gln
385 390 395 400

Leu Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn
405 410 415

Ala Ser Arg Cys Val Gly Arg Ile Gln Trp Ser Lys Leu Gln Val Phe
420 425 430

Asp Ala Arg Asp Cys Thr Thr Ala His Gly Met Phe Asn Tyr Ile Cys
435 440 445

Asn His Val Lys Tyr Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile

450 455 460

Thr Ile Phe Pro Gln Arg Thr Asp Gly Lys His Asp Phe Arg Val Trp
465 470 475 480

Asn Ser Gln Leu Ile Arg Tyr Ala Gly Tyr Lys Gln Pro Asp Gly Ser
485 490 495

Thr Leu Gly Asp Pro Ala Asn Val Gln Phe Thr Glu Ile Cys Ile Gln
500 505 510

Gln Gly Trp Lys Pro Pro Arg Gly Arg Phe Asp Val Leu Pro Leu Leu
515 520 525

Leu Gln Ala Asn Gly Asn Asp Pro Glu Leu Phe Gln Ile Pro Pro Glu
530 535 540

Leu Val Leu Glu Val Pro Ile Arg His Pro Lys Phe Glu Trp Phe Lys
545 550 555 560

Asp Leu Gly Leu Lys Trp Tyr Gly Leu Pro Ala Val Ser Asn Met Leu
565 570 575

Leu Glu Ile Gly Gly Leu Glu Phe Ser Ala Cys Pro Phe Ser Gly Trp
580 585 590

Tyr Met Gly Thr Glu Ile Gly Val Arg Asp Tyr Cys Asp Asn Ser Arg
595 600 605

Tyr Asn Ile Leu Glu Glu Val Ala Lys Lys Met Asn Leu Asp Met Arg
610 615 620

Lys Thr Ser Ser Leu Trp Lys Asp Gln Ala Leu Val Glu Ile Asn Ile
625 630 635 640

Ala Val Leu Tyr Ser Phe Gln Ser Asp Lys Val Thr Ile Val Asp His
645 650 655

His Ser Ala Thr Glu Ser Phe Ile Lys His Met Glu Asn Glu Tyr Arg
660 665 670

Cys Arg Gly Gly Cys Pro Ala Asp Trp Val Trp Ile Val Pro Pro Met
675 680 685

Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met Leu Asn Tyr Arg
690 695 700

Leu Thr Pro Ser Phe Glu Tyr Gln Pro Asp Pro Trp Asn Thr His Val
705 710 715 720

Trp Lys Gly Thr Asn Gly Thr Pro Thr Lys Arg Arg Ala Ile Gly Phe
725 730 735

Lys Lys Leu Ala Glu Ala Val Lys Phe Ser Ala Lys Leu Met Gly Gln
740 745 750

Ala Met Ala Lys Arg Val Lys Ala Thr Ile Leu Tyr Ala Thr Glu Thr
755 760 765

Gly Lys Ser Gln Ala Tyr Ala Lys Thr Leu Cys Glu Ile Phe Lys His
770 775 780

Ala Phe Asp Ala Lys Val Met Ser Met Glu Glu Tyr Asp Ile Val His
785 790 795 800

Leu Glu His Glu Thr Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn
805 810 815

Gly Asp Pro Pro Glu Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu
820 825 830

Met Arg His Pro Asn Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val
835 840 845

Arg Phe Asn Ser Val Ser Ser Tyr Ser Asp Ser Gln Lys Ser Ser Gly
850 855 860

Asp Gly Pro Asp Leu Arg Asp Asn Phe Glu Ser Ala Gly Pro Leu Ala
865 870 875 880

Asn Val Arg Phe Ser Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His
885 890 895

Phe Cys Ala Phe Gly His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly
900 905 910

Gly Glu Arg Ile Leu Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln
915 920 925

Glu Glu Ala Phe Arg Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys
930 935 940

Asp Val Phe Cys Val Gly Asp Asp Val Asn Ile Glu Lys Ala Asn Asn
945 950 955 960

Ser Leu Ile Ser Asn Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu
965 970 975

Thr Phe Val Ala Glu Ala Pro Glu Leu Thr Gln Gly Leu Ser Asn Val
980 985 990

His Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu
995 1000 1005

Gln Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr
1010 1015 1020

Asn Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val
1025 1030 1035 1040

Phe Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu
1045 1050 1055

Glu Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu Leu Glu
1060 1065 1070

Glu Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp Glu Leu
1075 1080 1085

Arg Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp
1090 1095 1100

Ile Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu
1105 1110 1115 1120

Ala Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly
1125 1130 1135

Leu Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr Ile Val
1140 1145 1150

Glu Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu
1155 1160 1165

Leu Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser

1170 1175 1180

Ser Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala Ile Val
1185 1190 1195 1200

Ser Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly Val Cys
1205 1210 1215

Ser Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro Cys Phe
1220 1225 1230

Val Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro
1235 1240 1245

Cys Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe
1250 1255 1260

Trp Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys
1265 1270 1275 1280

Pro Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile
1285 1290 1295

Tyr Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu
1300 1305 1310

Leu Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys Tyr Val
1315 1320 1325

Gln Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu
1330 1335 1340

Lys Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr Met Ala
1345 1350 1355 1360

Ala Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys
1365 1370 1375

Leu Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg Asp Asp
1380 1385 1390

Asn Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr Tyr Glu
1395 1400 1405

Val Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser
1410 1415 1420

Lys Lys Asp Thr Asp Glu Val Phe Ser Ser
1425 1430

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4780 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: Human NOS-SN gene, Nakane, et al,

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               FEBS Lett 316:175 (1993)

    (ix) FEATURE:
          (A) NAME/KEY: CDS
          (B) LOCATION: 431..4732

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GAGCGGACGG GCTCATGATG CCTCAGATCT GATCCGCATC TAACAGGCTG GCAATGAAGA       60

TACCCAGAGA ATAGTTCACA TCTATCATGC GTCACTTCTA GACACAGCCA TCAGACGCAT      120

CTCCTCCCCT TTCTGCCTGA CCTTAGGACA CGTCCCACCG CCTCTCTTGA CGTCTGCCTG      180

GTCAACCATC ACTTCCTTAG AGAATAAGGA GAGAGGCGGA TGCAGGAAAT CATGCCACCG      240

ACGGGCCACC AGCCATGAGT GGGTGACGCT GAGCTGACGT CAAAGACAGA GAGGGCTGAA      300

GCCTTGTCAG CACCTGTCAC CCCGGCTCCT GCTCTCCGTG TAGCCTGAAG CCTGGATCCT      360

CCTGGTGAAA TCATCTTGGC CTGATAGCAT TGTGAGGTCT TCAGACAGGA CCCCTCGGAA      420

GCTAGTTACC ATG GAG GAT CAC ATG TTC GGT GTT CAG CAA ATC CAG CCC         469
           Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro
             1               5                  10

AAT GTC ATT TCT GTT CGT CTC TTC AAG CGC AAA GTT GGG GGC CTG GGA        517
Asn Val Ile Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly
     15                  20                  25

TTT CTG GTG AAG GAG CGG GTC AGT AAG CCG CCC GTG ATC ATC TCT GAC        565
Phe Leu Val Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp
 30                  35                  40                  45

CTG ATT CGT GGG GGC GCC GCA GAG CAG AGT GGC CTC ATC CAG GCC GGA        613
Leu Ile Arg Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly
                 50                  55                  60

GAC ATC ATT CTT GCG GTC AAC GGC CGG CCC TTG GTG GAC CTG AGC TAT        661
Asp Ile Ile Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr
             65                  70                  75

GAC AGC GCC CTG GAG GTA CTC AGA GGC ATT GCC TCT GAG ACC CAC GTG        709
Asp Ser Ala Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val
         80                  85                  90

GTC CTC ATT CTG AGG GGC CCT GAA GGT TTC ACC ACG CAC CTG GAG ACC        757
Val Leu Ile Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr
     95                 100                 105

ACC TTT ACA GGT GAT GGG ACC CCC AAG ACC ATC CGG GTG ACA CAG CCC        805
Thr Phe Thr Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro
110                 115                 120                 125

CTG GGT CCC CCC ACC AAA GCC GTG GAT CTG TCC CAC CAG CCA CCG GCC        853
Leu Gly Pro Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala
                130                 135                 140

GGC AAA GAA CAG CCC CTG GCA GTG GAT GGG GCC TCG GGT CCC GGG AAT        901
Gly Lys Glu Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn
            145                 150                 155

GGG CCT CAG CAT GCC TAC GAT GAT GGG CAG GAG GCT GGC TCA CTC CCC        949
Gly Pro Gln His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro
        160                 165                 170
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CAT GCC AAC GGC TGG CCC CAG GCC CCC AGG CAG GAC CCC GCG AAG AAA          997
His Ala Asn Gly Trp Pro Gln Ala Pro Arg Gln Asp Pro Ala Lys Lys
    175                 180                 185

GCA ACC AGA GTC AGC CTC CAA GGC AGA GGG GAG AAC AAT GAA CTG CTC         1045
Ala Thr Arg Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu
190                 195                 200                 205

AAG GAG ATA GAG CCT GTG CTG AGC CTT CTC ACC AGT GGG AGC AGA GGG         1093
Lys Glu Ile Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly
                210                 215                 220

GTC AAG GGA GGG GCA CCT GCC AAG GCA GAG ATG AAA GAT ATG GGA ATC         1141
Val Lys Gly Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile
            225                 230                 235

CAG GTG GAC AGA GAT TTG GAC GGC AAG TCA CAC AAA CCT CTG CCC CTC         1189
Gln Val Asp Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu
        240                 245                 250

GGC GTG GAG AAC GAC CGA GTC TTC AAT GAC CTA TGG GGG AAG GGC AAT         1237
Gly Val Glu Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn
    255                 260                 265

GTG CCT GTC GTC CTC AAC AAC CCA TAT TCA GAG AAG GAG CAG CCC CCC         1285
Val Pro Val Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Pro
270                 275                 280                 285

ACC TCA GGA AAA CAG TCC CCC ACA AAG AAT GGC AGC CCC TCC AAG TGT         1333
Thr Ser Gly Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Ser Lys Cys
                290                 295                 300

CCA CGC TTC CTC AAG GTC AAG AAC TGG GAG ACT GAG GTG GTT CTC ACT         1381
Pro Arg Phe Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr
            305                 310                 315

GAC ACC CTC CAC CTT AAG AGC ACA TTG GAA ACG GGA TGC ACT GAG TAC         1429
Asp Thr Leu His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr
        320                 325                 330

ATC TGC ATG GGC TCC ATC ATG CAT CCT TCT CAG CAT GCA AGG AGG CCT         1477
Ile Cys Met Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro
    335                 340                 345

GAA GAC GTC CGC ACA AAA GGA CAG CTC TTC CCT CTC GCC AAA GAG TTT         1525
Glu Asp Val Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe
350                 355                 360                 365

ATT GAT CAA TAC TAT TCA TCA ATT AAA AGA TTT GGC TCC AAA GCC CAC         1573
Ile Asp Gln Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His
                370                 375                 380

ATG GAA AGG CTG GAA GAG GTG AAC AAA GAG ATC GAC ACC ACT AGC ACT         1621
Met Glu Arg Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr
            385                 390                 395

TAC CAG CTC AAG GAC ACA GAG CTC ATC TAT GGG GCC AAG CAC GCC TGG         1669
Tyr Gln Leu Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp
        400                 405                 410

CGG AAT GCC TCG CGC TGT GTG GGC AGG ATC CAG TGG TCC AAG CTG CAG         1717
Arg Asn Ala Ser Arg Cys Val Gly Arg Ile Gln Trp Ser Lys Leu Gln
    415                 420                 425

GTA TTC GAT GCC CGT GAC TGC ACC ACG GCC CAC GGG ATG TTC AAC TAC         1765
Val Phe Asp Ala Arg Asp Cys Thr Thr Ala His Gly Met Phe Asn Tyr
430                 435                 440                 445
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ATC TGT AAC CAT GTC AAG TAT GCC ACC AAC AAA GGG AAC CTC AGG TCT       1813
Ile Cys Asn His Val Lys Tyr Ala Thr Asn Lys Gly Asn Leu Arg Ser
                450                 455                 460

GCC ATC ACC ATA TTC CCC CAG AGG ACA GAC GGC AAG CAC GAC TTC CGA       1861
Ala Ile Thr Ile Phe Pro Gln Arg Thr Asp Gly Lys His Asp Phe Arg
            465                 470                 475

GTC TGG AAC TCC CAG CTC ATC CGC TAC GCT GGC TAC AAG CAC CGT GAC       1909
Val Trp Asn Ser Gln Leu Ile Arg Tyr Ala Gly Tyr Lys His Arg Asp
        480                 485                 490

GGC TCC ACC CTG GGG GAC CCA GCC AAT GTG CAG TTC ACA GAG ATA TGC       1957
Gly Ser Thr Leu Gly Asp Pro Ala Asn Val Gln Phe Thr Glu Ile Cys
    495                 500                 505

ATA CAG CAG GGC TGG AAA CCG CCT AGA GGC CGC TTC GAT GTC CTG CCG       2005
Ile Gln Gln Gly Trp Lys Pro Pro Arg Gly Arg Phe Asp Val Leu Pro
510                 515                 520                 525

CTC CTG CTT CAG GCC AAC GGC AAT GAC CCT GAG CTC TTC CAG ATT CCT       2053
Leu Leu Leu Gln Ala Asn Gly Asn Asp Pro Glu Leu Phe Gln Ile Pro
                530                 535                 540

CCA GAG CTG GTG TTG GAA CTT CCC ATC AGG CAC CCC AAG TTT GAG TGG       2101
Pro Glu Leu Val Leu Glu Leu Pro Ile Arg His Pro Lys Phe Glu Trp
            545                 550                 555

TTC AAG GAC CTG GCG CTG AAG TGG TAC GGC CTC CCC GCC GTG TCC AAC       2149
Phe Lys Asp Leu Ala Leu Lys Trp Tyr Gly Leu Pro Ala Val Ser Asn
        560                 565                 570

ATG CTC CTA GAG ATT GGC GGC CTG GAG TTC AGC GCC TGT CCC TTC AGT       2197
Met Leu Leu Glu Ile Gly Gly Leu Glu Phe Ser Ala Cys Pro Phe Ser
    575                 580                 585

GGC TGG TAC ATG GGC ACA GAG ATT GGT GTC CGC GAC TAC TGT GAC AAC       2245
Gly Trp Tyr Met Gly Thr Glu Ile Gly Val Arg Asp Tyr Cys Asp Asn
590                 595                 600                 605

TCC CGC TAC AAT ATC CTG GAG GAA GTG GCC AAG AAG ATG AAC TTA GAC       2293
Ser Arg Tyr Asn Ile Leu Glu Glu Val Ala Lys Lys Met Asn Leu Asp
                610                 615                 620

ATG AGG AAG ACG TCC TCC CTG TGG AAG GAC CAG GCG CTG GTG GAG ATC       2341
Met Arg Lys Thr Ser Ser Leu Trp Lys Asp Gln Ala Leu Val Glu Ile
            625                 630                 635

AAT ATC GCG GTT CTC TAT AGC TTC CAG AGT GAC AAA GTG ACC ATT GTT       2389
Asn Ile Ala Val Leu Tyr Ser Phe Gln Ser Asp Lys Val Thr Ile Val
        640                 645                 650

GAC CAT CAC TCC GCC ACC GAG TCC TTC ATT AAG CAC ATG GAG AAT GAG       2437
Asp His His Ser Ala Thr Glu Ser Phe Ile Lys His Met Glu Asn Glu
    655                 660                 665

TAC CGC TGC CGG GGG GGC TGC CCT GCC GAC TGG GTG TGG ATC GTG CCC       2485
Tyr Arg Cys Arg Gly Gly Cys Pro Ala Asp Trp Val Trp Ile Val Pro
670                 675                 680                 685

CCC ATG TCC GGA AGC ATC ACC CCT GTG TTC CAC CAG GAG ATG CTC AAC       2533
Pro Met Ser Gly Ser Ile Thr Pro Val Phe His Gln Glu Met Leu Asn
                690                 695                 700

TAC CGG CTC ACC CCC TCC TTC GAA TAC CAG CCT GAT CCC TGG AAC ACG       2581
Tyr Arg Leu Thr Pro Ser Phe Glu Tyr Gln Pro Asp Pro Trp Asn Thr
            705                 710                 715
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CAT GTC TGG AAA GGC ACC AAC GGG ACC CCC ACA AAG CGG CGA GCC ATC 2629
His Val Trp Lys Gly Thr Asn Gly Thr Pro Thr Lys Arg Arg Ala Ile
720 725 730

GGC TTC AAG AAG CTA GCA GAA GCT GTC AAG TTC TCG GCC AAG CTG ATG 2677
Gly Phe Lys Lys Leu Ala Glu Ala Val Lys Phe Ser Ala Lys Leu Met
735 740 745

GGG CAG GCT ATG GCC AAG AGG GTG AAA GCG ACC ATC CTC TAT GCC ACA 2725
Gly Gln Ala Met Ala Lys Arg Val Lys Ala Thr Ile Leu Tyr Ala Thr
750 755 760 765

GAG ACA GGC AAA TCG CAA GCT TAT GCC AAG ACC TTG TGT GAG ATC TTC 2773
Glu Thr Gly Lys Ser Gln Ala Tyr Ala Lys Thr Leu Cys Glu Ile Phe
770 775 780

AAA CAC GCC TTT GAT GCC AAG GTG ATG TCC ATG GAA GAA TAT GAC ATT 2821
Lys His Ala Phe Asp Ala Lys Val Met Ser Met Glu Glu Tyr Asp Ile
785 790 795

GTG CAC CTG GAA CAT GAA ACT CTG GTC CTT GTG GTC ACC AGC ACC TTT 2869
Val His Leu Glu His Glu Thr Leu Val Leu Val Val Thr Ser Thr Phe
800 805 810

GGC AAT GGA GAT CCC CCT GAG AAT GGG GAG AAA TTC GGC TGT GCT TTG 2917
Gly Asn Gly Asp Pro Pro Glu Asn Gly Glu Lys Phe Gly Cys Ala Leu
815 820 825

ATG GAA ATG AGG CAC CCC AAC TCT GTG CAG GAA GAA AGG AAG AGC TAC 2965
Met Glu Met Arg His Pro Asn Ser Val Gln Glu Glu Arg Lys Ser Tyr
830 835 840 845

AAG GTC CGA TTC AAC AGC GTC TCC TCC TAC TCT GAC TCC CAA AAA TCA 3013
Lys Val Arg Phe Asn Ser Val Ser Ser Tyr Ser Asp Ser Gln Lys Ser
850 855 860

TCA GGC GAT GGG CCC GAC CTC AGA GAC AAC TTT GAG AGT GCT GGA CCC 3061
Ser Gly Asp Gly Pro Asp Leu Arg Asp Asn Phe Glu Ser Ala Gly Pro
865 870 875

CTG GCC AAT GTG AGG TTC TCA GTT TTT GGC CTC GGC TCA CGA GCA TAC 3109
Leu Ala Asn Val Arg Phe Ser Val Phe Gly Leu Gly Ser Arg Ala Tyr
880 885 890

CCT CAC TTT TGC GCC TTC GGA CAC GCT GTG GAC ACC CTC CTG GAA GAA 3157
Pro His Phe Cys Ala Phe Gly His Ala Val Asp Thr Leu Leu Glu Glu
895 900 905

CTG GGA GGG GAG AGG ATC CTG AAG ATG AGG GAA GGG GAT GAG CTC TGT 3205
Leu Gly Gly Glu Arg Ile Leu Lys Met Arg Glu Gly Asp Glu Leu Cys
910 915 920 925

GGG CAG GAA GAG GCT TTC AGG ACC TGG GCC AAG AAG GTC TTC AAG GCA 3253
Gly Gln Glu Glu Ala Phe Arg Thr Trp Ala Lys Lys Val Phe Lys Ala
930 935 940

GCC TGT GAT GTC TTC TGT GTG GGA GAT GAT GTC AAC ATT GAA AAG GCC 3301
Ala Cys Asp Val Phe Cys Val Gly Asp Asp Val Asn Ile Glu Lys Ala
945 950 955

AAC AAT TCC CTC ATC AGC AAT GAT CGC AGC TGG AAG AGA AAC AAG TTC 3349
Asn Asn Ser Leu Ile Ser Asn Asp Arg Ser Trp Lys Arg Asn Lys Phe
960 965 970

CGC CTC ACC TTT GTG GCC GAA GCT CCA GAA CTC ACA CAA GGT CTA TCC 3397
Arg Leu Thr Phe Val Ala Glu Ala Pro Glu Leu Thr Gln Gly Leu Ser
975 980 985

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AAT GTC CAC AAA AAG CGA GTC TCA GCT GCC CGG CTC CTT AGC CGT CAA      3445
Asn Val His Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln
990                 995                 1000                1005

AAC CTC CAG AGC CCT AAA TCC AGT CGG TCA ACT ATC TTC GTG CGT CTC      34_3
Asn Leu Gln Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu
                1010                1015                1020

CAC ACC AAC GGG AGC CAG GAG CTG CAG TAC CAG CCT GGG GAC CAC CTG      3541
His Thr Asn Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu
            1025                1030                1035

GGT GTC TTC CCT GGC AAC CAC GAG GAC CTC GTG AAT GCC CTG ATC GAG      3589
Gly Val Phe Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu
        1040                1045                1050

CGG CTG GAG GAC GCG CCG CCT GTC AAC CAG ATG GTG AAA GTG GAA CTG      3637
Arg Leu Glu Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu
    1055                1060                1065

CTG GAG GAG CGG AAC ACG GCT TTA GGT GTC ATC AGT AAC TGG ACA GAC      3685
Leu Glu Glu Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp
1070                1075                1080                1085

GAG CTC CGC CTC CCG CCC TGC ACC ATC TTC CAG GCC TTC AAG TAC TAC      3733
Glu Leu Arg Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr
                1090                1095                1100

CTG GAC ATC ACC ACG CCA CCA ACG CCT CTG CAG CTG CAG CAG TTT GCC      3781
Leu Asp Ile Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala
            1105                1110                1115

TCC CTA GCT ACC AGC GAG AAG GAG AAG CAG CGT CTG CTG GTC CTC AGC      3829
Ser Leu Ala Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser
        1120                1125                1130

AAG GGT TTG CAG GAG TAC GAG GAA TGG AAA TGG GGC AAG AAC CCC ACC      3877
Lys Gly Leu Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr
    1135                1140                1145

ATC GTG GAG GTG CTG GAG GAG TTC CCA TCT ATC CAG ATG CCG GCC ACC      3925
Ile Val Glu Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr
1150                1155                1160                1165

CTG CTC CTG ACC CAG CTG TCC CTG CTG CAG CCC CGC TAC TAT TCC ATC      3973
Leu Leu Leu Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile
                1170                1175                1180

AGC TCC TCC CCA GAC ATG TAC CCT GAT GAA GTG CAC CTC ACT GTG GCC      4021
Ser Ser Ser Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala
            1185                1190                1195

ATC GTT TCC TAC CGC ACT CGA GAT GGA GAA GGA CCA ATT CAC CAC GGC      4069
Ile Val Ser Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly
        1200                1205                1210

GTA TGC TCC TCC TGG CTC AAC CGG ATA CAG GCT GAC GAA CTG GTC CCC      4117
Val Cys Ser Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro
    1215                1220                1225

TGT TTC GTG AGA GGA GCA CCC AGC TTC CAC CTG CCC CGG AAC CCC CAA      4165
Cys Phe Val Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln
1230                1235                1240                1245

GTC CCC TGC ATC CTC GTT GGA CCA GGC ACC GGC ATT GCC CCT TTC CGA      4213
Val Pro Cys Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg
                1250                1255                1260
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AGC TTC TGG CAA CAG CGG CAA TTT GAT ATC CAA CAC AAA GGA ATG AAC        4261
Ser Phe Trp Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn
            1265                1270                1275

CCC TGC CCC ATG GTC CTG GTC TTC GGG TGC CGG CAA TCC AAG ATA GAT        4309
Pro Cys Pro Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp
        1280                1285                1290

CAT ATC TAC AGG GAA GAG ACC CTG CAG GCC AAG AAC AAG GGG GTC TTC        4357
His Ile Tyr Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe
    1295                1300                1305

AGA GAG CTG TAC ACG GCT TAC TCC CGG GAG CCA GAC AAA CCA AAG AAG        4405
Arg Glu Leu Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys
1310                1315                1320                1325

TAC GTG CAG GAC ATC CTG CAG GAG CAG CTG GCG GAG TCT GTG TAC CGA        4453
Tyr Val Gln Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg
                1330                1335                1340

GCC CTG AAG GAG CAA GGG GGC CAC ATA TAC GTC TGT GGG GAC GTC ACC        4501
Ala Leu Lys Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr
            1345                1350                1355

ATG GCT GCT GAT GTC CTC AAA GCC ATC CAG CGC ATC ATG ACC CAG CAG        4549
Met Ala Ala Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln
        1360                1365                1370

GGG AAG CTC TCG GCA GAG GAC GCC GGC GTA TTC ATC AGC CGG ATG AGG        4597
Gly Lys Leu Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg
    1375                1380                1385

GAT GAC AAC CGA TAC CAT GAG GAT ATT TTT GGA GTC ACC CTG CGA ACG        4645
Asp Asp Asn Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr
1390                1395                1400                1405

ATC GAA GTG ACC AAC CGC CTT AGA TCT GAG TCC ATT GCC TTC ATT GAA        4693
Ile Glu Val Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu
                1410                1415                1420

GAG AGC AAA AAA GAC ACC GAT GAG GTT TTC AGC TCC TAACTGGACC             4739
Glu Ser Lys Lys Asp Thr Asp Glu Val Phe Ser Ser
            1425                1430

CTCTTGCCCA GCCGGCTGCA AGTTTGTAAG CGCGGGACAG A                          4780
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(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1433 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

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Met Glu Asp His Met Phe Gly Val Gln Gln Ile Gln Pro Asn Val Ile
1               5                   10                  15

Ser Val Arg Leu Phe Lys Arg Lys Val Gly Gly Leu Gly Phe Leu Val
            20                  25                  30

Lys Glu Arg Val Ser Lys Pro Pro Val Ile Ile Ser Asp Leu Ile Arg
        35                  40                  45
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Gly Gly Ala Ala Glu Gln Ser Gly Leu Ile Gln Ala Gly Asp Ile Ile
     50                  55                  60

Leu Ala Val Asn Gly Arg Pro Leu Val Asp Leu Ser Tyr Asp Ser Ala
65                  70                  75                  80

Leu Glu Val Leu Arg Gly Ile Ala Ser Glu Thr His Val Val Leu Ile
                85                  90                  95

Leu Arg Gly Pro Glu Gly Phe Thr Thr His Leu Glu Thr Thr Phe Thr
            100                 105                 110

Gly Asp Gly Thr Pro Lys Thr Ile Arg Val Thr Gln Pro Leu Gly Pro
        115                 120                 125

Pro Thr Lys Ala Val Asp Leu Ser His Gln Pro Pro Ala Gly Lys Glu
    130                 135                 140

Gln Pro Leu Ala Val Asp Gly Ala Ser Gly Pro Gly Asn Gly Pro Gln
145                 150                 155                 160

His Ala Tyr Asp Asp Gly Gln Glu Ala Gly Ser Leu Pro His Ala Asn
                165                 170                 175

Gly Trp Pro Gln Ala Pro Arg Gln Asp Pro Ala Lys Lys Ala Thr Arg
            180                 185                 190

Val Ser Leu Gln Gly Arg Gly Glu Asn Asn Glu Leu Leu Lys Glu Ile
        195                 200                 205

Glu Pro Val Leu Ser Leu Leu Thr Ser Gly Ser Arg Gly Val Lys Gly
    210                 215                 220

Gly Ala Pro Ala Lys Ala Glu Met Lys Asp Met Gly Ile Gln Val Asp
225                 230                 235                 240

Arg Asp Leu Asp Gly Lys Ser His Lys Pro Leu Pro Leu Gly Val Glu
                245                 250                 255

Asn Asp Arg Val Phe Asn Asp Leu Trp Gly Lys Gly Asn Val Pro Val
            260                 265                 270

Val Leu Asn Asn Pro Tyr Ser Glu Lys Glu Gln Pro Pro Thr Ser Gly
        275                 280                 285

Lys Gln Ser Pro Thr Lys Asn Gly Ser Pro Ser Lys Cys Pro Arg Phe
    290                 295                 300

Leu Lys Val Lys Asn Trp Glu Thr Glu Val Val Leu Thr Asp Thr Leu
305                 310                 315                 320

His Leu Lys Ser Thr Leu Glu Thr Gly Cys Thr Glu Tyr Ile Cys Met
                325                 330                 335

Gly Ser Ile Met His Pro Ser Gln His Ala Arg Arg Pro Glu Asp Val
            340                 345                 350

Arg Thr Lys Gly Gln Leu Phe Pro Leu Ala Lys Glu Phe Ile Asp Gln
        355                 360                 365

Tyr Tyr Ser Ser Ile Lys Arg Phe Gly Ser Lys Ala His Met Glu Arg
    370                 375                 380

Leu Glu Glu Val Asn Lys Glu Ile Asp Thr Thr Ser Thr Tyr Gln Leu
385                 390                 395                 400

Lys Asp Thr Glu Leu Ile Tyr Gly Ala Lys His Ala Trp Arg Asn Ala
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405 410 415

Ser Arg Cys Val Gly Arg Ile Gln Trp Ser Lys Leu Gln Val Phe Asp
420 425 430

Ala Arg Asp Cys Thr Thr Ala His Gly Met Phe Asn Tyr Ile Cys Asn
435 440 445

His Val Lys Tyr Ala Thr Asn Lys Gly Asn Leu Arg Ser Ala Ile Thr
450 455 460

Ile Phe Pro Gln Arg Thr Asp Gly Lys His Asp Phe Arg Val Trp Asn
465 470 475 480

Ser Gln Leu Ile Arg Tyr Ala Gly Tyr Lys His Arg Asp Gly Ser Thr
485 490 495

Leu Gly Asp Pro Ala Asn Val Gln Phe Thr Glu Ile Cys Ile Gln Gln
500 505 510

Gly Trp Lys Pro Pro Arg Gly Arg Phe Asp Val Leu Pro Leu Leu Leu
515 520 525

Gln Ala Asn Gly Asn Asp Pro Glu Leu Phe Gln Ile Pro Pro Glu Leu
530 535 540

Val Leu Glu Leu Pro Ile Arg His Pro Lys Phe Glu Trp Phe Lys Asp
545 550 555 560

Leu Ala Leu Lys Trp Tyr Gly Leu Pro Ala Val Ser Asn Met Leu Leu
565 570 575

Glu Ile Gly Gly Leu Glu Phe Ser Ala Cys Pro Phe Ser Gly Trp Tyr
580 585 590

Met Gly Thr Glu Ile Gly Val Arg Asp Tyr Cys Asp Asn Ser Arg Tyr
595 600 605

Asn Ile Leu Glu Glu Val Ala Lys Lys Met Asn Leu Asp Met Arg Lys
610 615 620

Thr Ser Ser Leu Trp Lys Asp Gln Ala Leu Val Glu Ile Asn Ile Ala
625 630 635 640

Val Leu Tyr Ser Phe Gln Ser Asp Lys Val Thr Ile Val Asp His His
645 650 655

Ser Ala Thr Glu Ser Phe Ile Lys His Met Glu Asn Glu Tyr Arg Cys
660 665 670

Arg Gly Gly Cys Pro Ala Asp Trp Val Trp Ile Val Pro Pro Met Ser
675 680 685

Gly Ser Ile Thr Pro Val Phe His Gln Glu Met Leu Asn Tyr Arg Leu
690 695 700

Thr Pro Ser Phe Glu Tyr Gln Pro Asp Pro Trp Asn Thr His Val Trp
705 710 715 720

Lys Gly Thr Asn Gly Thr Pro Thr Lys Arg Arg Ala Ile Gly Phe Lys
725 730 735

Lys Leu Ala Glu Ala Val Lys Phe Ser Ala Lys Leu Met Gly Gln Ala
740 745 750

Met Ala Lys Arg Val Lys Ala Thr Ile Leu Tyr Ala Thr Glu Thr Gly
755 760 765

Lys Ser Gln Ala Tyr Ala Lys Thr Leu Cys Glu Ile Phe Lys His Ala
770 775 780

Phe Asp Ala Lys Val Met Ser Met Glu Glu Tyr Asp Ile Val His Leu
785 790 795 800

Glu His Glu Thr Leu Val Leu Val Val Thr Ser Thr Phe Gly Asn Gly
805 810 815

Asp Pro Pro Glu Asn Gly Glu Lys Phe Gly Cys Ala Leu Met Glu Met
820 825 830

Arg His Pro Asn Ser Val Gln Glu Glu Arg Lys Ser Tyr Lys Val Arg
835 840 845

Phe Asn Ser Val Ser Ser Tyr Ser Asp Ser Gln Lys Ser Ser Gly Asp
850 855 860

Gly Pro Asp Leu Arg Asp Asn Phe Glu Ser Ala Gly Pro Leu Ala Asn
865 870 875 880

Val Arg Phe Ser Val Phe Gly Leu Gly Ser Arg Ala Tyr Pro His Phe
885 890 895

Cys Ala Phe Gly His Ala Val Asp Thr Leu Leu Glu Glu Leu Gly Gly
900 905 910

Glu Arg Ile Leu Lys Met Arg Glu Gly Asp Glu Leu Cys Gly Gln Glu
915 920 925

Glu Ala Phe Arg Thr Trp Ala Lys Lys Val Phe Lys Ala Ala Cys Asp
930 935 940

Val Phe Cys Val Gly Asp Asp Val Asn Ile Glu Lys Ala Asn Asn Ser
945 950 955 960

Leu Ile Ser Asn Asp Arg Ser Trp Lys Arg Asn Lys Phe Arg Leu Thr
965 970 975

Phe Val Ala Glu Ala Pro Glu Leu Thr Gln Gly Leu Ser Asn Val His
980 985 990

Lys Lys Arg Val Ser Ala Ala Arg Leu Leu Ser Arg Gln Asn Leu Gln
995 1000 1005

Ser Pro Lys Ser Ser Arg Ser Thr Ile Phe Val Arg Leu His Thr Asn
1010 1015 1020

Gly Ser Gln Glu Leu Gln Tyr Gln Pro Gly Asp His Leu Gly Val Phe
1025 1030 1035 1040

Pro Gly Asn His Glu Asp Leu Val Asn Ala Leu Ile Glu Arg Leu Glu
1045 1050 1055

Asp Ala Pro Pro Val Asn Gln Met Val Lys Val Glu Leu Leu Glu Glu
1060 1065 1070

Arg Asn Thr Ala Leu Gly Val Ile Ser Asn Trp Thr Asp Glu Leu Arg
1075 1080 1085

Leu Pro Pro Cys Thr Ile Phe Gln Ala Phe Lys Tyr Tyr Leu Asp Ile
1090 1095 1100

Thr Thr Pro Pro Thr Pro Leu Gln Leu Gln Gln Phe Ala Ser Leu Ala
1105 1110 1115 1120

Thr Ser Glu Lys Glu Lys Gln Arg Leu Leu Val Leu Ser Lys Gly Leu

1125 1130 1135

Gln Glu Tyr Glu Glu Trp Lys Trp Gly Lys Asn Pro Thr Ile Val Glu
1140 1145 1150

Val Leu Glu Glu Phe Pro Ser Ile Gln Met Pro Ala Thr Leu Leu Leu
1155 1160 1165

Thr Gln Leu Ser Leu Leu Gln Pro Arg Tyr Tyr Ser Ile Ser Ser Ser
1170 1175 1180

Pro Asp Met Tyr Pro Asp Glu Val His Leu Thr Val Ala Ile Val Ser
1185 1190 1195 1200

Tyr Arg Thr Arg Asp Gly Glu Gly Pro Ile His His Gly Val Cys Ser
1205 1210 1215

Ser Trp Leu Asn Arg Ile Gln Ala Asp Glu Leu Val Pro Cys Phe Val
1220 1225 1230

Arg Gly Ala Pro Ser Phe His Leu Pro Arg Asn Pro Gln Val Pro Cys
1235 1240 1245

Ile Leu Val Gly Pro Gly Thr Gly Ile Ala Pro Phe Arg Ser Phe Trp
1250 1255 1260

Gln Gln Arg Gln Phe Asp Ile Gln His Lys Gly Met Asn Pro Cys Pro
1265 1270 1275 1280

Met Val Leu Val Phe Gly Cys Arg Gln Ser Lys Ile Asp His Ile Tyr
1285 1290 1295

Arg Glu Glu Thr Leu Gln Ala Lys Asn Lys Gly Val Phe Arg Glu Leu
1300 1305 1310

Tyr Thr Ala Tyr Ser Arg Glu Pro Asp Lys Pro Lys Lys Tyr Val Gln
1315 1320 1325

Asp Ile Leu Gln Glu Gln Leu Ala Glu Ser Val Tyr Arg Ala Leu Lys
1330 1335 1340

Glu Gln Gly Gly His Ile Tyr Val Cys Gly Asp Val Thr Met Ala Ala
1345 1350 1355 1360

Asp Val Leu Lys Ala Ile Gln Arg Ile Met Thr Gln Gln Gly Lys Leu
1365 1370 1375

Ser Ala Glu Asp Ala Gly Val Phe Ile Ser Arg Met Arg Asp Asp Asn
1380 1385 1390

Arg Tyr His Glu Asp Ile Phe Gly Val Thr Leu Arg Thr Ile Glu Val
1395 1400 1405

Thr Asn Arg Leu Arg Ser Glu Ser Ile Ala Phe Ile Glu Glu Ser Lys
1410 1415 1420

Lys Asp Thr Asp Glu Val Phe Ser Ser
1425 1430

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 256 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: EPO-1 HRE element

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

```
GAACTGAAAC CACCAATATG ACTCTTGGCT TTTCTGTTTT CTGGGAACCT CCAAATCCCC    60
TGGCTCTGTC CCACTCCTGG CAGCAGTGCA GCAGGTCCAG GTCCGGGAAA TGAGGGGTGG   120
AGGGGGCTGG GCCCTACGTG CTGTCTCACA CAGCCTGTCT GACCTCTCGA CCTACCGGCC   180
TAGGCCACAA GCTCTGCCTA CGCTGGTCAA TAAGGTGTCT CCATTCAAGG CCTCACCGCA   240
GTAAGGCAGC TGCCAA                                                   256
```

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: 42 bp EPO 3' hypoxia response enhancer element (Madan, *et al*, PNAS 90:3928, 1993)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

```
GGGCCCTACG TGCTGTCTCA CACAGCCTGT CTGACCTCTC GA                       42
```

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 86 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: 86 nucleotide fragment from αMHC promoter

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
GTCCCAGCAG ATGACTCCAA ATTTAGGCAG CAGGCACGTG GAATGAGCTA TAAAGGGGCT        60

GGAGCGCTGA GAGCTGTCAG ACCGAG                                             86
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2423 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: mouse catalase gene GenBank #L25069

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 88..1671

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
ATTGCCTTCT CCGGGTGGAG ACCAGACCGC TGCGTCCGTC CCTGCTGTCT CACGTTCCGC        60

AGCTCTGCAG CTCCGCAATC CTACACC ATG TCG GAC AGT CGG GAC CCA GCC           111
                              Met Ser Asp Ser Arg Asp Pro Ala
                                1               5

AGC GAC CAG ATG AAG CAG TGG AAG GAG CAG CGG GCC TCG CAG AGA CCT         159
Ser Asp Gln Met Lys Gln Trp Lys Glu Gln Arg Ala Ser Gln Arg Pro
     10                  15                  20

GAT GTC CTG ACC ACC GGA GGC GGG AAC CCA ATA GGA GAT AAA CTT AAT         207
Asp Val Leu Thr Thr Gly Gly Gly Asn Pro Ile Gly Asp Lys Leu Asn
 25                  30                  35                  40

ATC ATG ACC GCG GGG TCC CGA GGG CCC CTC CTC GTT CAG GAT GTG GTT         255
Ile Met Thr Ala Gly Ser Arg Gly Pro Leu Leu Val Gln Asp Val Val
                 45                  50                  55

TTC ACT GAC GAG ATG GCA CAC TTT GAC AGA GAG CGG ATT CCT GAG AGA         303
Phe Thr Asp Glu Met Ala His Phe Asp Arg Glu Arg Ile Pro Glu Arg
             60                  65                  70

GTG GTA CAC GCA AAA GGA GCA GGT GCT TTT GGA TAC TTT GAG GTC ACC         351
Val Val His Ala Lys Gly Ala Gly Ala Phe Gly Tyr Phe Glu Val Thr
         75                  80                  85

CAC GAT ATC ACC AGA TAC TCC AAG GGA AAG GTG TTT GAG CAT ATT GGA         399
His Asp Ile Thr Arg Tyr Ser Lys Gly Lys Val Phe Glu His Ile Gly
     90                  95                 100

AAG AGG ACC CCT ATT GCC GTT CGG TTC TCC ACA GTC GCT GGA GAG TCA         447
Lys Arg Thr Pro Ile Ala Val Arg Phe Ser Thr Val Ala Gly Glu Ser
105                 110                 115                 120

GGC TCA GCT GAC ACA GTT CGT GAC CCT CGG GGG TTT GCA GTG AAA TTT         495
Gly Ser Ala Asp Thr Val Arg Asp Pro Arg Gly Phe Ala Val Lys Phe
                125                 130                 135
```

```
TAC ACT GAA GAT GGT AAC TGG GAT CTT GTG GGA AAC AAC ACC CCT ATT      543
Tyr Thr Glu Asp Gly Asn Trp Asp Leu Val Gly Asn Asn Thr Pro Ile
            140                 145                 150

TTC TTC ATC AGG GAT GCC ATA TTG TTT CCA TCC TTT ATC CAT AGC CAG      591
Phe Phe Ile Arg Asp Ala Ile Leu Phe Pro Ser Phe Ile His Ser Gln
        155                 160                 165

AAG AGA AAC CCA CAG ACT CAC CTG AAG GAT CCT GAC ATG GTC TGG GAC      639
Lys Arg Asn Pro Gln Thr His Leu Lys Asp Pro Asp Met Val Trp Asp
    170                 175                 180

TTC TGG AGT CTT CGT CCC GAG TCT CTC CAT CAG GTT TCT TTC TTG TTC      687
Phe Trp Ser Leu Arg Pro Glu Ser Leu His Gln Val Ser Phe Leu Phe
185                 190                 195                 200

AGT GAC CGA GGG ATT CCC GAT GGT CAC CGG CAC ATG AAT GGC TAT GGA      735
Ser Asp Arg Gly Ile Pro Asp Gly His Arg His Met Asn Gly Tyr Gly
                205                 210                 215

TCA CAC ACC TTC AAG TTG GTT AAT GCA GAT GGA GAG GCA GTC TAT TGC      783
Ser His Thr Phe Lys Leu Val Asn Ala Asp Gly Glu Ala Val Tyr Cys
            220                 225                 230

AAG TTC CAT TAC AAG ACC GAC CAG GGC ATC AAA AAC TTG CCT GTT GGA      831
Lys Phe His Tyr Lys Thr Asp Gln Gly Ile Lys Asn Leu Pro Val Gly
        235                 240                 245

GAG GCA GGA AGG CTT GCT CAG GAA GAT CCG GAT TAT GGC CTC CGA GAT      879
Glu Ala Gly Arg Leu Ala Gln Glu Asp Pro Asp Tyr Gly Leu Arg Asp
    250                 255                 260

CTT TTC AAT GCC ATC GCC AAT GGC AAT TAC CCG TCC TGG ACG TTT TAC      927
Leu Phe Asn Ala Ile Ala Asn Gly Asn Tyr Pro Ser Trp Thr Phe Tyr
265                 270                 275                 280

ATC CAG GTC ATG ACT TTT AAG GAG GCA GAA ACT TTC CCA TTT AAT CCA      975
Ile Gln Val Met Thr Phe Lys Glu Ala Glu Thr Phe Pro Phe Asn Pro
                285                 290                 295

TTT GAT CTG ACC AAG GTT TGG CCT CAC AAG GAC TAC CCT CTT ATA CCA     1023
Phe Asp Leu Thr Lys Val Trp Pro His Lys Asp Tyr Pro Leu Ile Pro
            300                 305                 310

GTT GGC AAA GTG GTT TTA AAC AAA AAT CCA GTT AAT TAC TTT GCT GAA     1071
Val Gly Lys Val Val Leu Asn Lys Asn Pro Val Asn Tyr Phe Ala Glu
        315                 320                 325

GTT GAA CAG ATG GCT TTT GAC CCA AGC AAT ATG CCC CCT GGC ATC GAG     1119
Val Glu Gln Met Ala Phe Asp Pro Ser Asn Met Pro Pro Gly Ile Glu
    330                 335                 340

CCC AGC CCT GAC AAA AAG CTT CAG GGC CGC CTT TTT GCC TAC CCG GAC     1167
Pro Ser Pro Asp Lys Lys Leu Gln Gly Arg Leu Phe Ala Tyr Pro Asp
345                 350                 355                 360

ACT CAC CGC CAC CGC CTG GGA CCC AAC TAT CTG CAG ATA CCT GTG AAC     1215
Thr His Arg His Arg Leu Gly Pro Asn Tyr Leu Gln Ile Pro Val Asn
                365                 370                 375

TGT CCC TAC CGC GCT CGA GTG GCC AAC TAC CAG CGT GAT GGC CCC ATG     1263
Cys Pro Tyr Arg Ala Arg Val Ala Asn Tyr Gln Arg Asp Gly Pro Met
            380                 385                 390

TGC ATG CAT GAC AAC CAG GGT GGT GCC CCC AAC TAT TAC CCC AAC AGC     1311
Cys Met His Asp Asn Gln Gly Gly Ala Pro Asn Tyr Tyr Pro Asn Ser
        395                 400                 405
```

TTC AGC GCA CCA GAG CAG CAG CGC TCA GCC CTG GAG CAC AGC GTC CAG 1359
Phe Ser Ala Pro Glu Gln Gln Arg Ser Ala Leu Glu His Ser Val Gln
410 415 420

TGC GCT GTA GAT GTG AAA CGC TTC AAC AGT GCT AAT GAA GAC AAT GTC 1407
Cys Ala Val Asp Val Lys Arg Phe Asn Ser Ala Asn Glu Asp Asn Val
425 430 435 440

ACT CAG GTG CGG ACA TTC TAC ACA AAG GTG TTG AAT GAG GAG GAG AGG 1455
Thr Gln Val Arg Thr Phe Tyr Thr Lys Val Leu Asn Glu Glu Glu Arg
445 450 455

AAA CGC CTG TGT GAG AAC ATT GCC GGC CAC CTG AAG GAC GCT CAG CTT 1503
Lys Arg Leu Cys Glu Asn Ile Ala Gly His Leu Lys Asp Ala Gln Leu
460 465 470

TTC ATT CAG AAG AAA GCG GTC AAG AAT TTC ACT GAC GTC CAC CCT GAC 1551
Phe Ile Gln Lys Lys Ala Val Lys Asn Phe Thr Asp Val His Pro Asp
475 480 485

TAT GGG GCC CGC ATC CAG GCT CTT CTG GAC AAG TAC AAC GCT GAG AAG 1599
Tyr Gly Ala Arg Ile Gln Ala Leu Leu Asp Lys Tyr Asn Ala Glu Lys
490 495 500

CCT AAG AAC GCA ATT CAC ACC TAC ACG CAG GCC GGC TCT CAC ATG GCT 1647
Pro Lys Asn Ala Ile His Thr Tyr Thr Gln Ala Gly Ser His Met Ala
505 510 515 520

GCG AAG GGA AAA GCT AAC CTG TAACTCCGGT GCTCAGCCTC CGCTGAGGAG 1698
Ala Lys Gly Lys Ala Asn Leu
525

ACCTCTCGTG AAGCCGAGCC TGAGGATCAC CTGTAATCAA CGCTGGATGG ATTCTCCCCC 1758

GCCGGAGCGC AGACTCACGC TGATGACTTT AAAACGATAA TCCGGGCTTC TAGAGTGAAT 1818

GATAACCATG CTTTTGATGC CGTTTCCTGA AGGGAAATGA AAGGTTAGGG CTTAGCAATC 1878

ATTTAACAGA AACATGGATC TAATAGGACT TCTGTTTGGA TTATTCATTT AAATGACTAC 1938

ATTTAAAATG ATTACAAGAA AGGTGTTCTA GCCAGAAACA TGACTTGATT AGACAAGATA 1998

AAAATCTTGG CGAGAATAGT GTATTCTCCT ATTACCTCAT GGTCTGGTAT ATATACAATA 2058

CAACACACAT ACCACACACA CACACACATG CAATACACAC ACTACACACA CATACACACA 2118

CTCACACACA CTCATACACA CACATGAAGA GATGATAAAG ATGGCCCACT CAGAATTTTT 2178

TTTTTATTTT TCTAAGGTCC TTATAAGCAA AACCATACTT GCATCATGTC TTCCAAAAGT 2238

AACTTTAGCA CTGTTGAAAC TTAATGTTTA TTCCTGTGCT GTGCGGTGCT GTGCTGTGCT 2298

GTGCTGTGCA GCTAATCAGA TTCTTGTTTT TTCCCACTTG GATTATGTTG ATGCTAATAC 2358

GCAGTGATTT CACATAGGAT GATTTGTACT TGCTTACATT TTTACAATAA AATGATCTAC 2418

ATGGA 2423

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 527 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Ser Asp Ser Arg Asp Pro Ala Ser Asp Gln Met Lys Gln Trp Lys
  1               5                  10                  15

Glu Gln Arg Ala Ser Gln Arg Pro Asp Val Leu Thr Thr Gly Gly Gly
             20                  25                  30

Asn Pro Ile Gly Asp Lys Leu Asn Ile Met Thr Ala Gly Ser Arg Gly
         35                  40                  45

Pro Leu Leu Val Gln Asp Val Val Phe Thr Asp Glu Met Ala His Phe
     50                  55                  60

Asp Arg Glu Arg Ile Pro Glu Arg Val Val His Ala Lys Gly Ala Gly
 65                  70                  75                  80

Ala Phe Gly Tyr Phe Glu Val Thr His Asp Ile Thr Arg Tyr Ser Lys
                 85                  90                  95

Gly Lys Val Phe Glu His Ile Gly Lys Arg Thr Pro Ile Ala Val Arg
            100                 105                 110

Phe Ser Thr Val Ala Gly Glu Ser Gly Ser Ala Asp Thr Val Arg Asp
        115                 120                 125

Pro Arg Gly Phe Ala Val Lys Phe Tyr Thr Glu Asp Gly Asn Trp Asp
    130                 135                 140

Leu Val Gly Asn Asn Thr Pro Ile Phe Phe Ile Arg Asp Ala Ile Leu
145                 150                 155                 160

Phe Pro Ser Phe Ile His Ser Gln Lys Arg Asn Pro Gln Thr His Leu
                165                 170                 175

Lys Asp Pro Asp Met Val Trp Asp Phe Trp Ser Leu Arg Pro Glu Ser
            180                 185                 190

Leu His Gln Val Ser Phe Leu Phe Ser Asp Arg Gly Ile Pro Asp Gly
        195                 200                 205

His Arg His Met Asn Gly Tyr Gly Ser His Thr Phe Lys Leu Val Asn
    210                 215                 220

Ala Asp Gly Glu Ala Val Tyr Cys Lys Phe His Tyr Lys Thr Asp Gln
225                 230                 235                 240

Gly Ile Lys Asn Leu Pro Val Gly Glu Ala Gly Arg Leu Ala Gln Glu
                245                 250                 255

Asp Pro Asp Tyr Gly Leu Arg Asp Leu Phe Asn Ala Ile Ala Asn Gly
            260                 265                 270

Asn Tyr Pro Ser Trp Thr Phe Tyr Ile Gln Val Met Thr Phe Lys Glu
        275                 280                 285

Ala Glu Thr Phe Pro Phe Asn Pro Phe Asp Leu Thr Lys Val Trp Pro
    290                 295                 300

His Lys Asp Tyr Pro Leu Ile Pro Val Gly Lys Val Val Leu Asn Lys
305                 310                 315                 320

Asn Pro Val Asn Tyr Phe Ala Glu Val Glu Gln Met Ala Phe Asp Pro
                325                 330                 335

Ser Asn Met Pro Pro Gly Ile Glu Pro Ser Pro Asp Lys Lys Leu Gln
            340                 345                 350
```

```
Gly Arg Leu Phe Ala Tyr Pro Asp Thr His Arg His Arg Leu Gly Pro
        355                 360                 365

Asn Tyr Leu Gln Ile Pro Val Asn Cys Pro Tyr Arg Ala Arg Val Ala
    370                 375                 380

Asn Tyr Gln Arg Asp Gly Pro Met Cys Met His Asp Asn Gln Gly Gly
385                 390                 395                 400

Ala Pro Asn Tyr Tyr Pro Asn Ser Phe Ser Ala Pro Glu Gln Gln Arg
                405                 410                 415

Ser Ala Leu Glu His Ser Val Gln Cys Ala Val Asp Val Lys Arg Phe
            420                 425                 430

Asn Ser Ala Asn Glu Asp Asn Val Thr Gln Val Arg Thr Phe Tyr Thr
        435                 440                 445

Lys Val Leu Asn Glu Glu Glu Arg Lys Arg Leu Cys Glu Asn Ile Ala
    450                 455                 460

Gly His Leu Lys Asp Ala Gln Leu Phe Ile Gln Lys Lys Ala Val Lys
465                 470                 475                 480

Asn Phe Thr Asp Val His Pro Asp Tyr Gly Ala Arg Ile Gln Ala Leu
                485                 490                 495

Leu Asp Lys Tyr Asn Ala Glu Lys Pro Lys Asn Ala Ile His Thr Tyr
            500                 505                 510

Thr Gln Ala Gly Ser His Met Ala Ala Lys Gly Lys Ala Asn Leu
        515                 520                 525
```

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 969 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA to mRNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: human manganese superoxide dismutase
EMBL #X59445

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 61..729

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

```
TGGCTTCGGC AGCGGCTTCA GCAGATCGGC GGCATCAGCG GTAGCACCAG CACTAGCAGC      60

ATG TTG AGC CGG GCA GTG TGC GGC ACC AGC AGG CAG CTG GCT CCG GCT       108
Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala
  1               5                  10                  15

TTG GGG TAT CTG GGC TCC AGG CAG AAG CAC AGC CTC CCC GAC CTG CCC       156
Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Leu Pro Asp Leu Pro
```

```
                 20                            25                            30

TAC GAC TAC GGC GCC CTG GAA CCT CAC ATC AAC GCG CAG ATC ATG CAG        204
Tyr Asp Tyr Gly Ala Leu Glu Pro His Ile Asn Ala Gln Ile M t Gln
            35                  40                  45

CTG CAC CAC AGC AAG CAC CAC GCG GCC TAC GTG AAC AAC CTG AAC GTC        252
Leu His His Ser Lys His His Ala Ala Tyr Val Asn Asn Leu Asn Val
        50                  55                  60

AAC GAG GAG AAG TAC CAG GAG GCG TTG GCC AAG GGA GAT GTT ACA GCC        300
Asn Glu Glu Lys Tyr Gln Glu Ala Leu Ala Lys Gly Asp Val Thr Ala
 65                 70                  75                      80

CAG ATA GCT CTT CAG CCT GCA CTG AAG TTC AAT GGT GGT GGT CAT ATC        348
Gln Ile Ala Leu Gln Pro Ala Leu Lys Phe Asn Gly Gly Gly His Ile
                85                  90                  95

AAT CAT AGC ATT TTC TGG ACA AAC CTC AGC CCT AAC GGT GGT GGA GAA        396
Asn His Ser Ile Phe Trp Thr Asn Leu Ser Pro Asn Gly Gly Gly Glu
            100                 105                 110

CCC AAA GGG GAG TTG CTG GAA GCC ATC AAA CGT GAC TTT GGT TCC TTT        444
Pro Lys Gly Glu Leu Leu Glu Ala Ile Lys Arg Asp Phe Gly Ser Phe
        115                 120                 125

GAC AAG TTT AAG GAG AAG CTG ACG GCT GCA TCT GTT GGT GTC CAA GGC        492
Asp Lys Phe Lys Glu Lys Leu Thr Ala Ala Ser Val Gly Val Gln Gly
    130                 135                 140

TCA GGT TGG GGT TGG CTT GGT TTC AAT AAG GAA CGG GGA CAC TTA CAA        540
Ser Gly Trp Gly Trp Leu Gly Phe Asn Lys Glu Arg Gly His Leu Gln
145                 150                 155                     160

ATT GCT GCT TGT CCA AAT CAG GAT CCA CTG CAA GGA ACA ACA GGC CTT        588
Ile Ala Ala Cys Pro Asn Gln Asp Pro Leu Gln Gly Thr Thr Gly Leu
                165                 170                 175

ATT CCA CTG CTG GGG ATT GAT GTG TGG GAG CAC GCT TAC TAC CTT CAG        636
Ile Pro Leu Leu Gly Ile Asp Val Trp Glu His Ala Tyr Tyr Leu Gln
            180                 185                 190

TAT AAA AAT GTC AGG CCT GAT TAT CTA AAA GCT ATT TGG AAT GTA ATC        684
Tyr Lys Asn Val Arg Pro Asp Tyr Leu Lys Ala Ile Trp Asn Val Ile
        195                 200                 205

AAC TGG GAG AAT GTA ACT GAA AGA TAC ATG GCT TGC AAA AAG TAAACCACGA     736
Asn Trp Glu Asn Val Thr Glu Arg Tyr Met Ala Cys Lys Lys
    210                 215                 220

TCGTTATGCT GAGTATGTTA AGCTCTTTAT GACTGTTTTT GTAGTGGTAT AGAGTACTGC      796

AGAATACAGT AAGCTGCTCT ATTGTAGCAT TTCTTGATGT TGCTTAGTCA CTTATTTCAT      856

AAACAACTTA ATGTTCTGAA TAATTTCTTA CTAAACATTT TGTTATTGGG CAAGTGATTG      916

AAAATAGTAA ATGCTTTGTG TGATTGAAAA AAAAAAAAAA AAAAAAAAAA AAA             969
```

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 222 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

```
Met Leu Ser Arg Ala Val Cys Gly Thr Ser Arg Gln Leu Ala Pro Ala
1               5                   10                  15

Leu Gly Tyr Leu Gly Ser Arg Gln Lys His Ser Leu Pro Asp Leu Pro
            20                  25                  30

Tyr Asp Tyr Gly Ala Leu Glu Pro His Ile Asn Ala Gln Ile Met Gln
        35                  40                  45

Leu His His Ser Lys His His Ala Ala Tyr Val Asn Asn Leu Asn Val
    50                  55                  60

Asn Glu Glu Lys Tyr Gln Glu Ala Leu Ala Lys Gly Asp Val Thr Ala
65                  70                  75                  80

Gln Ile Ala Leu Gln Pro Ala Leu Lys Phe Asn Gly Gly Gly His Ile
                85                  90                  95

Asn His Ser Ile Phe Trp Thr Asn Leu Ser Pro Asn Gly Gly Gly Glu
            100                 105                 110

Pro Lys Gly Glu Leu Leu Glu Ala Ile Lys Arg Asp Phe Gly Ser Phe
        115                 120                 125

Asp Lys Phe Lys Glu Lys Leu Thr Ala Ala Ser Val Gly Val Gln Gly
    130                 135                 140

Ser Gly Trp Gly Trp Leu Gly Phe Asn Lys Glu Arg Gly His Leu Gln
145                 150                 155                 160

Ile Ala Ala Cys Pro Asn Gln Asp Pro Leu Gln Gly Thr Thr Gly Leu
                165                 170                 175

Ile Pro Leu Leu Gly Ile Asp Val Trp Glu His Ala Tyr Tyr Leu Gln
            180                 185                 190

Tyr Lys Asn Val Arg Pro Asp Tyr Leu Lys Ala Ile Trp Asn Val Ile
        195                 200                 205

Asn Trp Glu Asn Val Thr Glu Arg Tyr Met Ala Cys Lys Lys
    210                 215                 220
```

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 691 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: human enolase gene (EMBL #X56832) fragment containinig nucleotides -628 to +63

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 629..691

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

```
CCTGGGGGTG GAGGTAGTAA AGGGTGAGCA TGGTATTGGC TTGGAGGAAG TGGGGGACAT       60
TTCTGCTTTT TTTCCTCCTG GGACTGGAGA TGCTTGAAAA AGCTGGGGGA AGGGGCGGCT      120
GGAGCAAGCA GATGGGACAA ACTCTGGGAA CACCGAAGGA TCTAGGGAAA GGAGGCTGTG      180
AGGAGGGCAG CAGGGATGGA TAGAAAAGGG CAGCTAGAGC TGGAACCTGA TAGGGAATTG      240
GGGGCCCAAG GAGATTTCGG AGCAGGAAAA TGAGAACCAG AAAGGATTTG AAGGCCACCA      300
GCCATGGAGA ACAGACTGCT TGACCAGAGG GGTGGAAGGA GAAGGCCTAA GTGGAGGCTT      360
GGGGGAGGTG GGGGCTTGGT GAGCGGTGGC ATCCCAGGAG CTATAGATAA GAGGCCCCTG      420
GATTCTTAGG ATGGGAGGGT GGAATAAGAG CTGTTCTGAG TGGGGGAGGG GGCTGCGCCT      480
GCCTCTTTGG TCTGTGACCT TTTTGTAGGG TATTTTTAGC TCCAGCACCT GCCTTCTTGG      540
AGTGGGGAAG AATCTTAAAG GGCAAGGGAT TTCTGGTTCC TTAAGAGATC AACTGTCTAC      600
ACTCACTCAC ACCTCCTGTC CTGCAGCC ATG GCC ATG CAG AAA ATC TTT GCC         652
                               Met Ala Met Gln Lys Ile Phe Ala
                                 1               5

CGG GAA ATC TTG GAC TCC AGG GGC AAC CCC ACG GTG GAG                    691
Arg Glu Ile Leu Asp Ser Arg Gly Asn Pro Thr Val Glu
     10                 15                  20
```

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

```
Met Ala Met Gln Lys Ile Phe Ala Arg Glu Ile Leu Asp Ser Arg Gly
  1               5                  10                  15

Asn Pro Thr Val Glu
            20
```

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: PKM/ENO3 consensus sequence

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GAGAGGCGGG CTNNCTG 17

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 786 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: -760 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

AAGCTTGTGG CTTCTTCTCC TTACTCTTCC TCCTTGGTGT CTCTATGTTA GAGGGCCGTT 60

AGCATCTGCT GGGGCCTGGT CGCATTCACC CTGCTCTGCC ACTCACTGGC TGTGTGACTC 120

TGGACAAATT AACTTCTCTG GACCTGGCAG TTTCTCCTCT CTACAATGAG AATACTGGAG 180

AGTCCTTATC TTATGGGTTG CTACAGAATT AAGTGACATC TCACACACAA CACACTTCCT 240

ACAGTCCCTG TTACACGCTA AAAGTACTCA ACTAGCTTCG GATACGTCAT CAGCAACCAC 300

CCCACGGGTT ACTGTGATGC TGCACAATTA TTAAGCCCTG GCTGCTACAG AGTTGTAACC 360

TGTCTGCACT TCCAACCGGC GCCGCAAGCA GCATTCCCAG TCCCGCTTTC ACCCGCGCGC 420

TAACGGCTCA GGTTCGAGTA CAGGACAGGA GGGAGGGGAG CTGTGCACAC GGCGGAGGCG 480

CACGGCGTGG GCACCCAGCA CCCGGTACAC TGTGTCCTCC CGCTGCACCC AGCCCCTTCA 540

GCCCGAGGCG TCCCCGAGGC GCAAGTGGGC CGCCTTCAGG GAACTGACCG CCCGCGGCCC 600

GTGTGCAGAG CCGGGTGCGC CCGGCCCAGT GCGCGCGGCC GGGTGTTTCG CTTGGAGCCG 660

CAAGTGACTT CTAGCGCGGG GCGTGTGCAG GCACGGCCGG GGCGGGGCTT TTGCACTCGT 720

CCCGGCTCTT TCTAGCTATA AACACTGCTT GCCGCGCTGC ACTCCACCAC GCCTCCTCCA 780

AGTCCC 786

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 366 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: -345 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
TAACGGCTCA GGTTCGAGTA CAGGACAGGA GGGAGGGGAG CTGTGCACAC GGCGGAGGCG     60
CACGGCGTGG GCACCCAGCA CCCGGTACAC TGTGTCCTCC CGCTGCACCC AGCCCCTTCA    120
GCCCGAGGCG TCCCCGAGGC GCAAGTGGGC CGCCTTCAGG GAACTGACCG CCCGCGGCCC    180
GTGTGCAGAG CCGGGTGCGC CCGGCCCAGT GCGCGCGGCC GGGTGTTTCG CTTGGAGCCG    240
CAAGTGACTT CTAGCGCGGG GCGTGTGCAG GCACGGCCGG GGCGGGGCTT TTGCACTCGT    300
CCCGGCTCTT TCTAGCTATA AACACTGCTT GCCGCGCTGC ACTCCACCAC GCCTCCTCCA    360
AGTCCC                                                               366
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 184 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: -163 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
GTGCAGAGCC GGGTGCGCCC GGCCCAGTGC GCGCGGCCGG GTGTTTCGCT TGGAGCCGCA     60
AGTGACTTCT AGCGCGGGGC GTGTGCAGGC ACGGCCGGGG CGGGGCTTTT GCACTCGTCC    120
CGGCTCTTTC TAGCTATAAA CACTGCTTGC CGCGCTGCAC TCCACCACGC CTCCTCCAAG    180
TCCC                                                                 184
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: -90 MTAIIa promoter fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
GCGGGGCGTG TGCAGGCACG GCCGGGGCGG GGCTTTTGCA CTCGTCCCGG CTCTTTCTAG     60
CTATAAACAC TGCTTGCCGC GCTGCACTCC ACCACGCCTC CTCCAAGTCC C            111
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1643 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(C) INDIVIDUAL ISOLATE: TNF cDNA HSTNFR (EMBL Accession #X01394)

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 153..851

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
GCAGAGGACC AGCTAAGAGG GAGAGAAGCA ACTACAGACC CCCCCTGAAA ACAACCCTCA      60

GACGCCACAT CCCCTGACAA GCTGCCAGGC AGGTTCTCTT CCTCTCACAT ACTGACCCAC     120

GGCTCCACCC TCTCTCCCCT GGAAAGGACA CC ATG AGC ACT GAA AGC ATG ATC       173
                                    Met Ser Thr Glu Ser Met Ile
                                     1               5

CGG GAC GTG GAG CTG GCC GAG GAG GCG CTC CCC AAG AAG ACA GGG GGG       221
Arg Asp Val Glu Leu Ala Glu Glu Ala Leu Pro Lys Lys Thr Gly Gly
        10                  15                  20

CCC CAG GGC TCC AGG CGG TGC TTG TTC CTC AGC CTC TTC TCC TTC CTG       269
Pro Gln Gly Ser Arg Arg Cys Leu Phe Leu Ser Leu Phe Ser Phe Leu
    25                  30                  35

ATC GTG GCA GGC GCC ACC ACG CTC TTC TGC CTG CTG CAC TTT GGA GTG       317
Ile Val Ala Gly Ala Thr Thr Leu Phe Cys Leu Leu His Phe Gly Val
 40                  45                  50                  55

ATC GGC CCC CAG AGG GAA GAG TTC CCC AGG GAC CTC TCT CTA ATC AGC       365
Ile Gly Pro Gln Arg Glu Glu Phe Pro Arg Asp Leu Ser Leu Ile Ser
                60                  65                  70

CCT CTG GCC CAG GCA GTC AGA TCA TCT TCT CGA ACC CCG AGT GAC AAG       413
Pro Leu Ala Gln Ala Val Arg Ser Ser Ser Arg Thr Pro Ser Asp Lys
            75                  80                  85

CCT GTA GCC CAT GTT GTA GCA AAC CCT CAA GCT GAG GGG CAG CTC CAG       461
Pro Val Ala His Val Val Ala Asn Pro Gln Ala Glu Gly Gln Leu Gln
        90                  95                 100

TGG CTG AAC CGC CGG GCC AAT GCC CTC CTG GCC AAT GGC GTG GAG CTG       509
Trp Leu Asn Arg Arg Ala Asn Ala Leu Leu Ala Asn Gly Val Glu Leu
    105                 110                 115

AGA GAT AAC CAG CTG GTG GTG CCA TCA GAG GGC CTG TAC CTC ATC TAC       557
Arg Asp Asn Gln Leu Val Val Pro Ser Glu Gly Leu Tyr Leu Ile Tyr
120                 125                 130                 135

TCC CAG GTC CTC TTC AAG GGC CAA GGC TGC CCC TCC ACC CAT GTG CTC       605
Ser Gln Val Leu Phe Lys Gly Gln Gly Cys Pro Ser Thr His Val Leu
```

```
                    140                 145                 150

CTC ACC CAC ACC ATC AGC CGC ATC GCC GTC TCC TAC CAG ACC AAG GTC        653
Leu Thr His Thr Ile Ser Arg Ile Ala Val Ser Tyr Gln Thr Lys Val
            155                 160                 165

AAC CTC CTC TCT GCC ATC AAG AGC CCC TGC CAG AGG GAG ACC CCA GAG        701
Asn Leu Leu Ser Ala Ile Lys Ser Pro Cys Gln Arg Glu Thr Pro Glu
        170                 175                 180

GGG GCT GAG GCC AAG CCC TGG TAT GAG CCC ATC TAT CTG GGA GGG GTC        749
Gly Ala Glu Ala Lys Pro Trp Tyr Glu Pro Ile Tyr Leu Gly Gly Val
    185                 190                 195

TTC CAG CTG GAG AAG GGT GAC CGA CTC AGC GCT GAG ATC AAT CGG CCC        797
Phe Gln Leu Glu Lys Gly Asp Arg Leu Ser Ala Glu Ile Asn Arg Pro
200                 205                 210                 215

GAC TAT CTC GAC TTT GCC GAG TCT GGG CAG GTC TAC TTT GGG ATC ATT        845
Asp Tyr Leu Asp Phe Ala Glu Ser Gly Gln Val Tyr Phe Gly Ile Ile
                220                 225                 230

GCC CTG TGAGGAGGAC GAACATCCAA CCTTCCCAAA CGCCTCCCCT GCCCCAATCC        901
Ala Leu

CTTTATTACC CCCTCCTTCA GACACCCTCA ACCTCTTCTG GCTCAAAAAG AGAATTGGGG      961

GCTTAGGGTC GGAACCCAAG CTTAGAACTT TAAGCAACAA GACCACCACT TCGAAACCTG     1021

GGATTCAGGA ATGTGTGGCC TGCACAGTGA ATTGCTGGCA ACCACTAAGA ATTCAAACTG     1081

GGGCCTCCAG AACTCACTGG GGCCTACAGC TTTGATCCCT GACATCTGGA ATCTGGAGAC     1141

CAGGGAGCCT TTGGTTCTGG CCAGAATGCT GCAGGACTTG AGAAGACCTC ACCTAGAAAT     1201

TGACACAAGT GGACCTTAGG CCTTCCTCTC TCCAGATGTT TCCAGACTTC CTTGAGACAC     1261

GGAGCCCAGC CCTCCCCATG GAGCCAGCTC CCTCTATTTA TGTTTGCACT TGTGATTATT     1321

TATTATTTAT TTATTATTTA TTTATTTACA GATGAATGTA TTTATTTGGG AGACCGGGGT     1381

ATCCTGGGGG ACCCAATGTA GGAGCTGCCT TGGCTCAGAC ATGTTTTCCG TGAAAACGGA     1441

GCTGAACAAT AGGCTGTTCC CATGTAGCCC CCTGGCCTCT GTGCCTTCTT TTGATTATGT     1501

TTTTTAAAAT ATTTATCTGA TTAAGTTGTC TAAACAATGC TGATTTGGTG ACCAACTGTC     1561

ACTCATTGCT GAGCCTCTGC TCCCCAGGGG AGTTGTGTCT GTAATCGCCC TACTATTCAG     1621

TGGCGAGAAA TAAAGTTTGC TT                                              1643
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 233 amino acids
  (B) TYPE: amino acid
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Met Ser Thr Glu Ser Met Ile Arg Asp Val Glu Leu Ala Glu Glu Ala
 1               5                  10                  15
```

Leu Pro Lys Lys Thr Gly Gly Pro Gln Gly Ser Arg Arg Cys Leu Phe
20 25 30

Leu Ser Leu Ph Ser Phe Leu Ile Val Ala Gly Ala Thr Thr Leu Phe
35 40 45

Cys Leu Leu His Phe Gly Val Ile Gly Pro Gln Arg Glu Glu Phe Pro
50 55 60

Arg Asp Leu Ser Leu Ile Ser Pro Leu Ala Gln Ala Val Arg Ser Ser
65 70 75 80

Ser Arg Thr Pro Ser Asp Lys Pro Val Ala His Val Val Ala Asn Pro
85 90 95

Gln Ala Glu Gly Gln Leu Gln Trp Leu Asn Arg Arg Ala Asn Ala Leu
100 105 110

Leu Ala Asn Gly Val Glu Leu Arg Asp Asn Gln Leu Val Val Pro Ser
115 120 125

Glu Gly Leu Tyr Leu Ile Tyr Ser Gln Val Leu Phe Lys Gly Gln Gly
130 135 140

Cys Pro Ser Thr His Val Leu Leu Thr His Thr Ile Ser Arg Ile Ala
145 150 155 160

Val Ser Tyr Gln Thr Lys Val Asn Leu Leu Ser Ala Ile Lys Ser Pro
165 170 175

Cys Gln Arg Glu Thr Pro Glu Gly Ala Glu Ala Lys Pro Trp Tyr Glu
180 185 190

Pro Ile Tyr Leu Gly Gly Val Phe Gln Leu Glu Lys Gly Asp Arg Leu
195 200 205

Ser Ala Glu Ile Asn Arg Pro Asp Tyr Leu Asp Phe Ala Glu Ser Gly
210 215 220

Gln Val Tyr Phe Gly Ile Ile Ala Leu
225 230

IT IS CLAIMED:

1. A chimeric gene, comprising

a hypoxia response enhancer element, a tissue-specific promoter heterologous to the element, and a therapeutic gene,

wherein said promoter is operably linked to said therapeutic gene and said element is effective to modulate expression of said therapeutic gene.

2. A chimeric gene of claim 1, wherein said promoter is a cardiac-specific promoter.

3. A chimeric gene of claim 2, wherein said promoter is selected from the group consisting of $\alpha$-$MHC_{5.5}$ promoter, $\alpha$-$MHC_{87}$ promoter, and human cardiac actin promoter.

4. A chimeric gene of claim 1, wherein said promoter is a kidney-specific promoter.

5. A chimeric gene of claim 4, wherein said promoter is a renin promoter.

6. A chimeric gene of claim 1, wherein said promoter is a brain-specific promoter.

7. A chimeric gene of claim 6, wherein said promoter is selected from the group consisting of aldolase C promoter, and tyrosine hydroxylase promoter.

8. A chimeric gene of claim 1, wherein said promoter is a vascular endothelium-specific promoter.

9. A chimeric gene of claim 8, wherein said promoter is selected from the group consisting of Et-1 promoter and vonWillebrand factor promoter.

10. A chimeric gene of claim 1, wherein said hypoxia response enhancer element is selected from the group consisting of erythropoietin HRE element (HREE1), pyruvate kinase (PKM) HRE element, enolase 3 (ENO3) HRE element, endothelin-1 (ET-1) HRE element and metallothionein II (MTII) HRE element.

11. A chimeric gene of claim 10, wherein said HRE element has a sequence contained in SEQ ID NO:35.

12. A chimeric gene of claim 1, wherein said therapeutic gene is selected from the group consisting of nitric oxide synthase (NOS), Bcl-2, superoxide dismutase (SOD), and catalase.

13. An expression vector, comprising the chimeric gene of any of claims 1-12.

14. An expression vector of claim 13, wherein said expression vector is a plasmid.

15. An expression vector of claim 13, wherein said expression vector is an adenovirus vector.

16. An expression vector of claim 13, wherein said expression vector is a retrovirus vector.

17. A method of reducing ischemic injury to a cell exposed to hypoxic conditions, comprising

introducing into said cell a chimeric gene of any of claims 1-12,

wherein exposing the cell to hypoxic conditions increases expression of said therapeutic gene and wherein expression of said therapeutic gene is effective to reduce ischemic injury to the cell.

18. A method of claim 17, wherein said cell is a vascular endothelium cell and said promoter is a vascular endothelium-specific promoter.

19. A method of reducing ischemic injury to a cell exposed to hypoxic conditions, comprising

introducing into said cell a chimeric gene containing a hypoxia response enhancer element, a therapeutic gene, and a tissue-specific promoter operably linked to said therapeutic gene, where said element is effective to modulate expression of said therapeutic gene,

wherein exposing the cell to hypoxic conditions increases expression of said therapeutic gene and wherein expression of said therapeutic gene is effective to reduce ischemic injury to the cell.

20. A hypoxia response enhancer (HRE) element consisting of a sequence derived from SEQ ID NO:35.

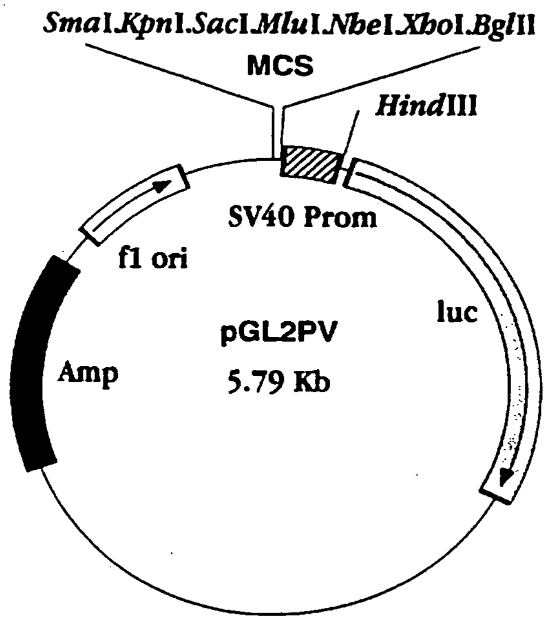

SmaI.KpnI.SacI.MluI.NheI.XhoI.BglII
MCS
HindIII
SV40 Prom
f1 ori
luc
pGL2PV
5.79 Kb
Amp

FIG. 1A

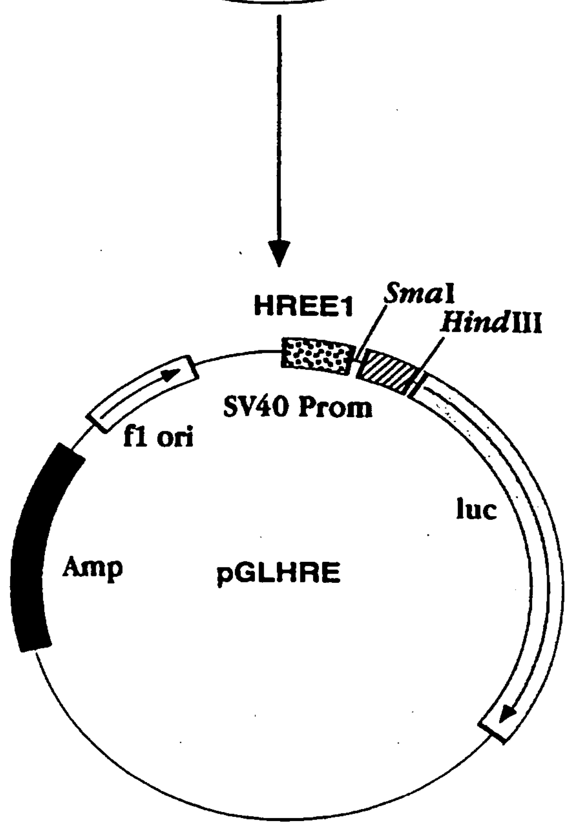
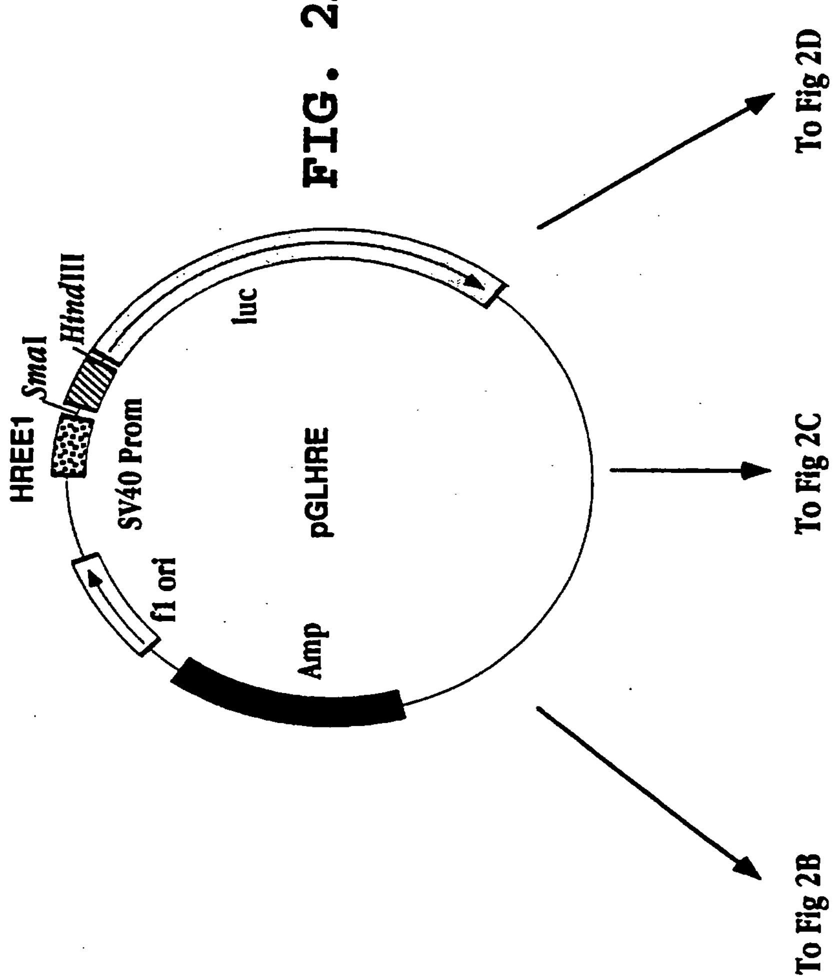

HREE1
SmaI
HindIII
SV40 Prom
f1 ori
luc
Amp
pGLHRE

FIG. 1B

FIG. 2A


HREE1
SmaI
HindIII
luc
SV40 Prom
pGLHRE
f1 ori
Amp
To Fig 2D
To Fig 2C
To Fig 2B

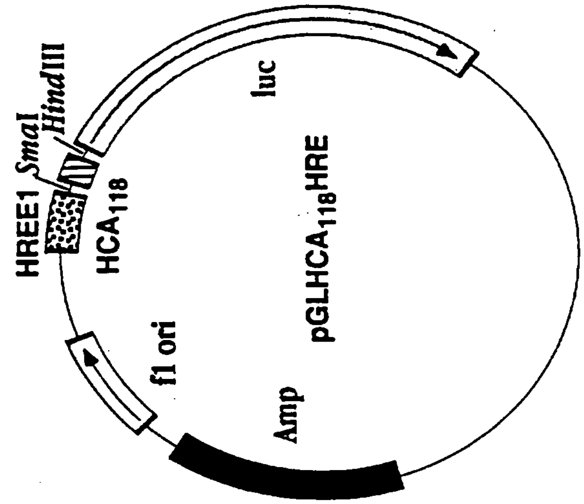



FIG. 2D

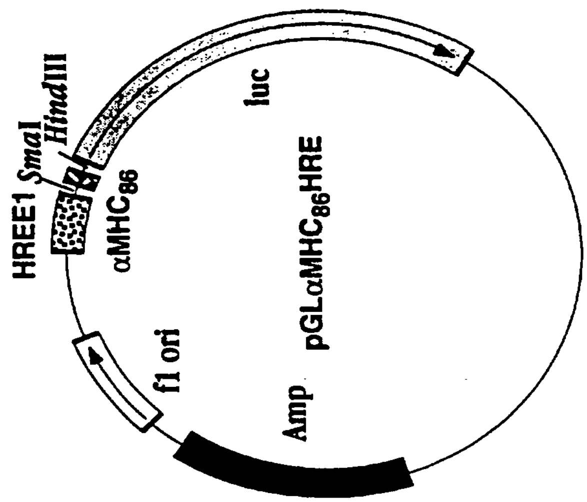



FIG. 2C

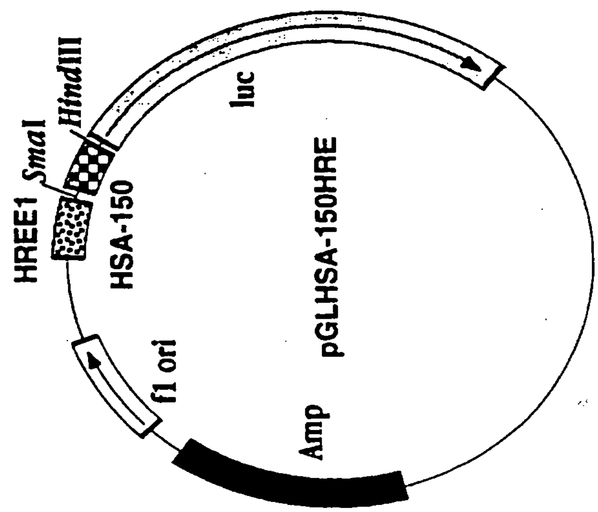



FIG. 2B

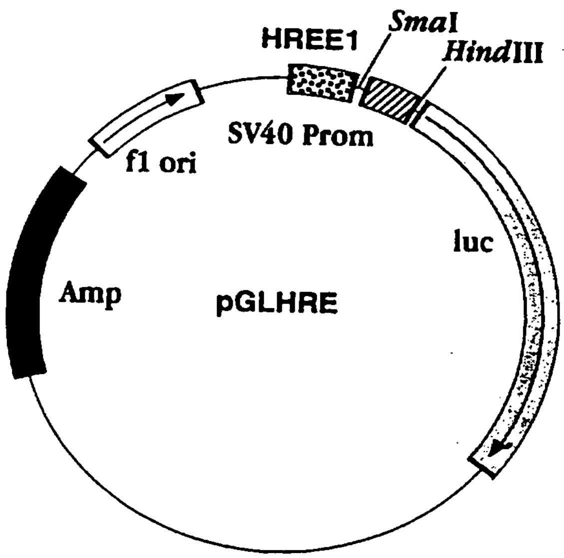

HREE1
SmaI
HindIII
SV40 Prom
f1 ori
luc
Amp
pGLHRE

FIG. 3A

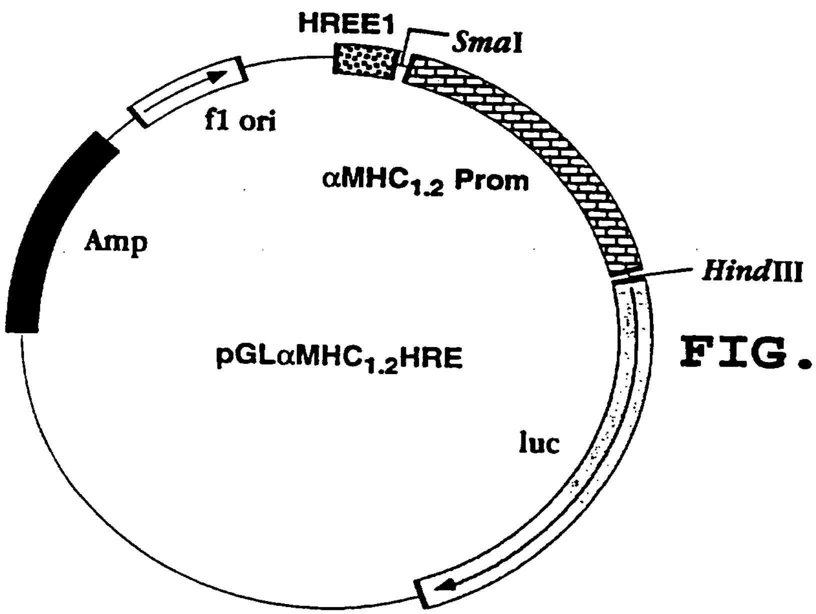

HREE1
SmaI
f1 ori
αMHC1.2 Prom
Amp
HindIII
pGLαMHC1.2HRE
luc

FIG. 3B

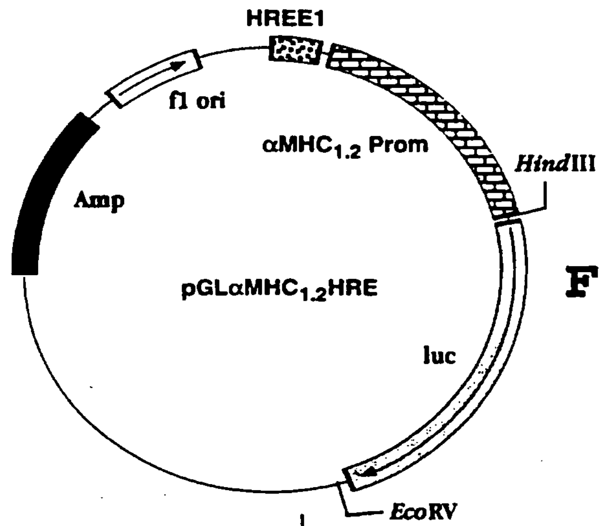

HREE1
f1 ori
αMHC1.2 Prom
HindIII
Amp
pGLαMHC1.2HRE
luc
EcoRV

FIG. 4A

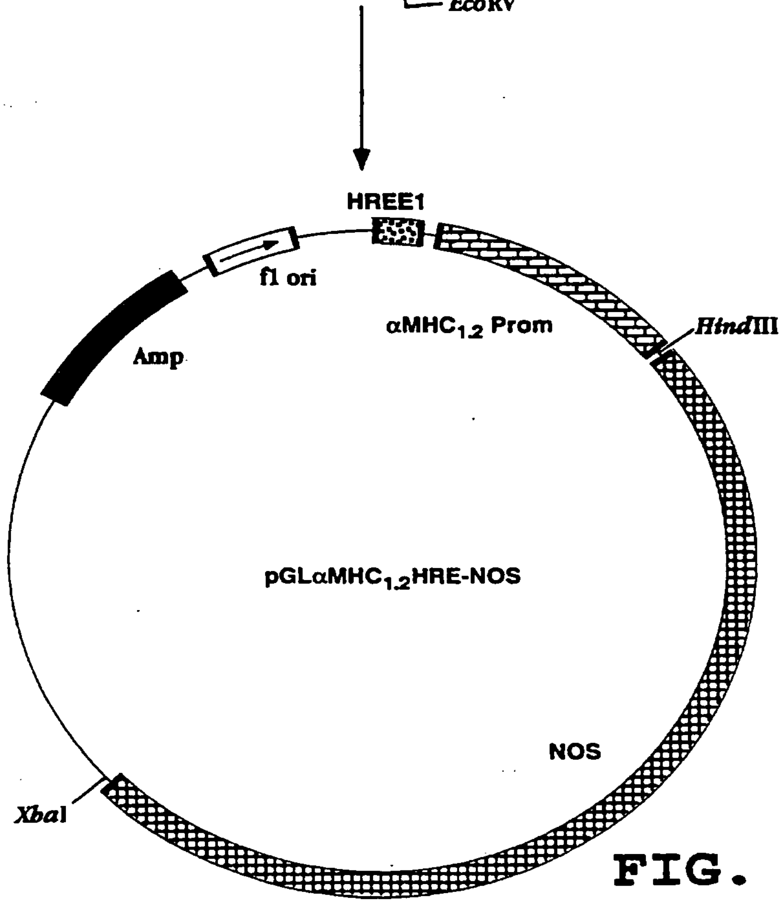

HREE1
f1 ori
αMHC1.2 Prom
HindIII
Amp
pGLαMHC1.2HRE-NOS
NOS
XbaI

FIG. 4B

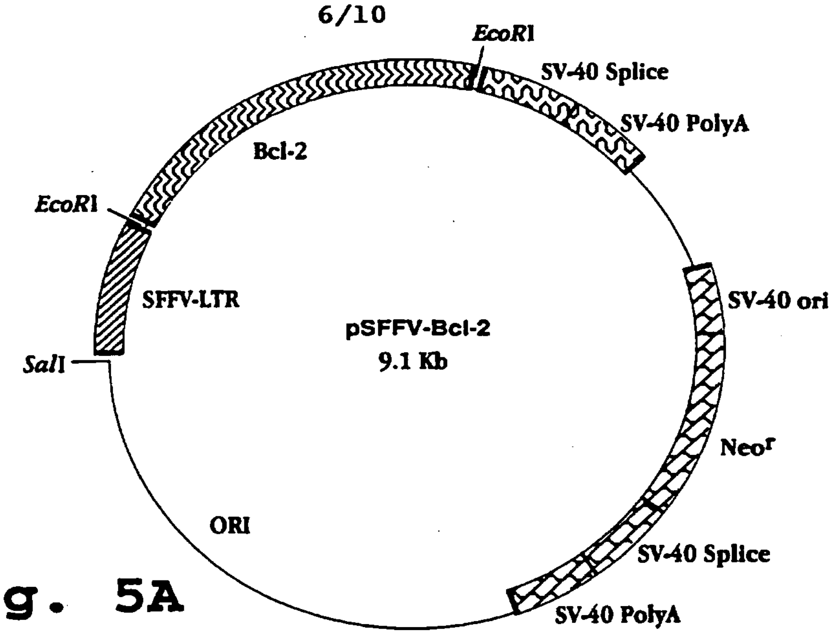

EcoRI
SV-40 Splice
SV-40 PolyA
Bcl-2
EcoRI
SFFV-LTR
pSFFV-Bcl-2
9.1 Kb
SV-40 ori
SalI
Neo$^r$
ORI
SV-40 Splice
SV-40 PolyA

Fig. 5A

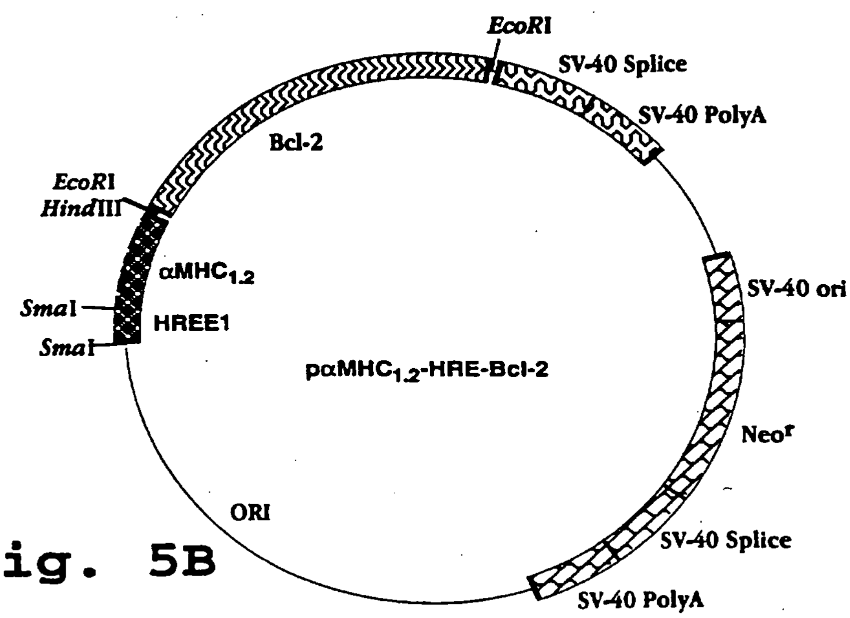

EcoRI
SV-40 Splice
SV-40 PolyA
Bcl-2
EcoRI
HindIII
$\alpha$MHC$_{1.2}$
SV-40 ori
SmaI
HREE1
SmaI
p$\alpha$MHC$_{1.2}$-HRE-Bcl-2
Neo$^r$
ORI
SV-40 Splice
SV-40 PolyA

Fig. 5B

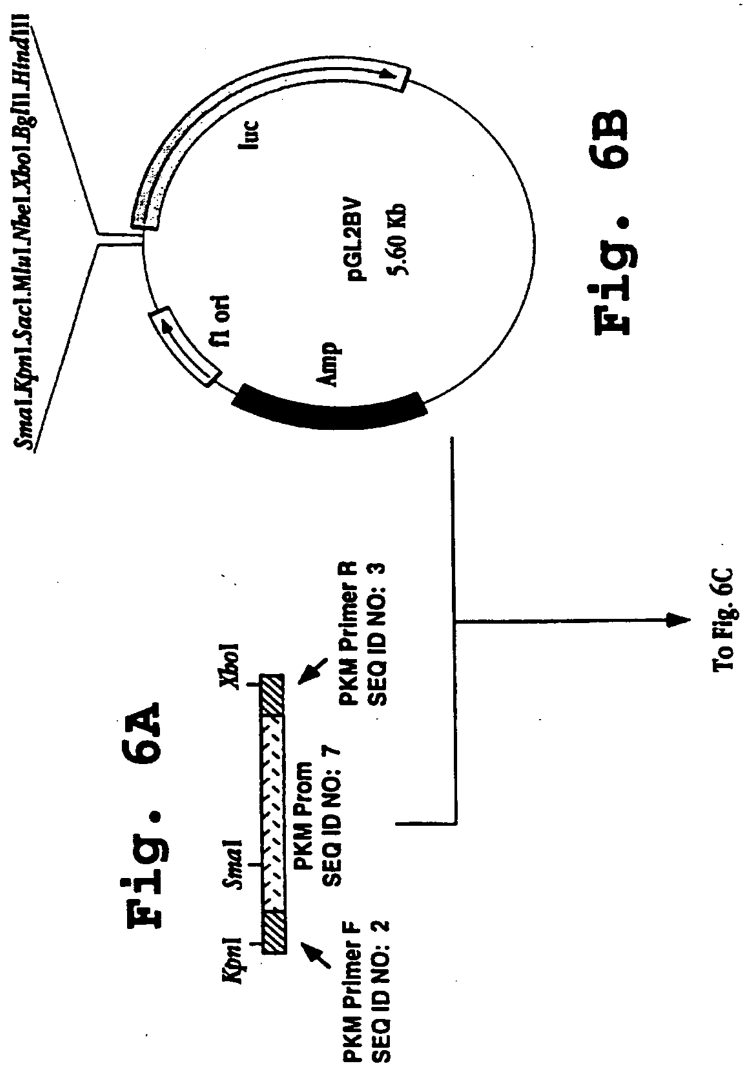

SmaI.KpnI.SacI.MluI.NheI.XhoI.BglII.HindIII
luc
pGL2BV
5.60 Kb
f1 ori
Amp
KpnI
SmaI
XhoI
PKM Prom
SEQ ID NO: 7
PKM Primer F
SEQ ID NO: 2
PKM Primer R
SEQ ID NO: 3
To Fig. 6C

Fig. 6A

Fig. 6B

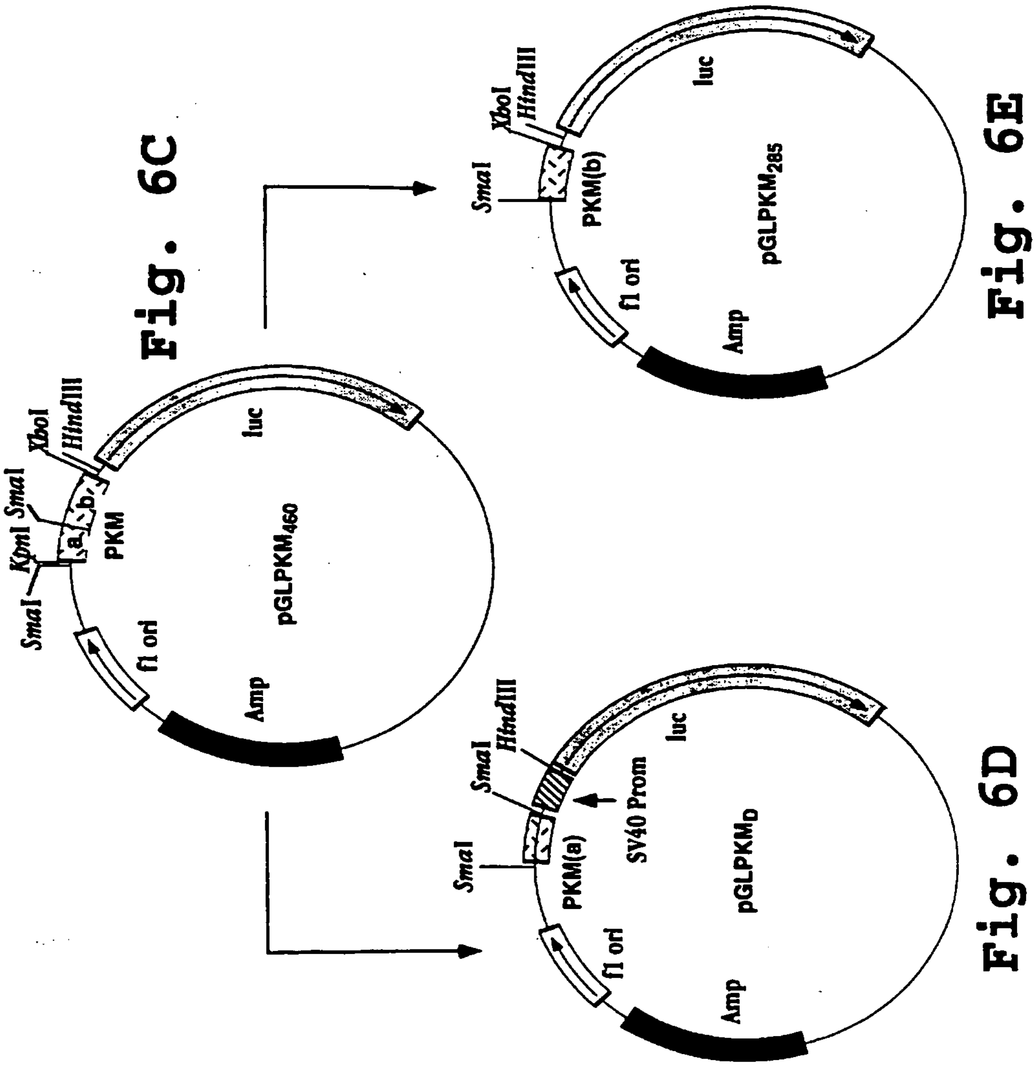



Fig. 6C

Fig. 6D

Fig. 6E

Fig. 7A

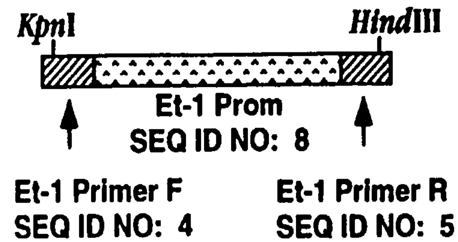
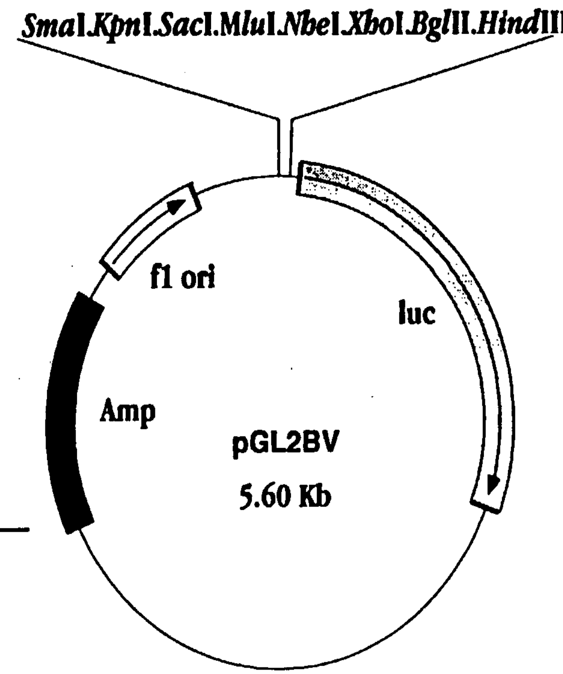
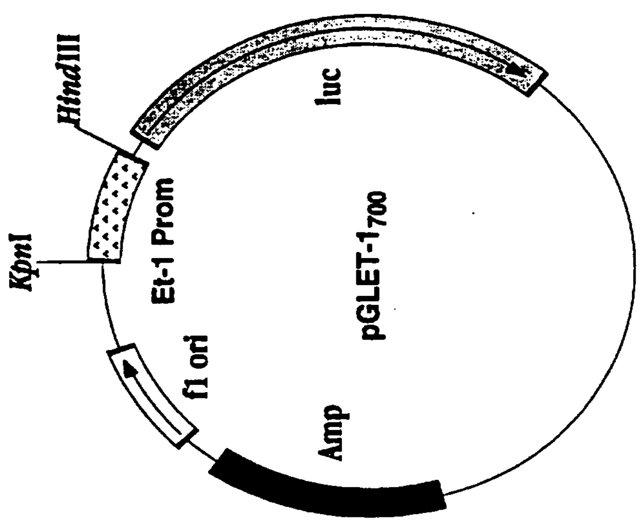



Fig. 7B




To Fig. 7C

Fig. 7C



# INTERNATIONAL SEARCH REPORT

Inte   onal Application No
PCT/IB 95/00996

**A. CLASSIFICATION OF SUBJECT MATTER**
IPC 6 C12N15/11 C12N15/67

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | PROCEEDINGS OF THE ACADEMY OF SCIENCE, vol. 90, 1993 pages 3928-3932, A. MADAN ET AL. 'A 24-bp sequence 3' to the human EPO gene contains a hypoxia-responsive transcriptional enhancer' *see the whole article* --- | 1-20 |
| X | PROCEEDINGS OF THE ACADEMY OF SCIENCE, vol. 90, 1993 pages 4304-4308, G.L. WANG ET AL. 'General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia' *see the whole article* --- | 1-20 |
| | -/-- | |

[X] Further documents are listed in the continuation of box C.

[ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 1 April 1996 | 22.04.96 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Marie, A |

Inte .onal Application No
PCT/IB 95/00996

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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